REMARKS

In the Office Action dated august 5, 2008, claims 1-16 are pending, claims 5-7 and 13-16 are withdrawn form consideration, and claims 1-4 and 8-12 stand rejected. The rejection is made final. Reconsideration is requested at least for the reasons discussed hereinbelow.

The above amendments are submitted to more particularly point out and distinctly claim the subject matter regarded as invention. Claims 1, 2 and 3 have been combined with claim 10 in amended claims 1, 2 and 3.

Claims 1-4 and 8-12 are rejected under 35 U.S.C. §112, first paragraph. The Examiner states that claims are enabled at least for treatment of portal hypertension and its bleeding complications. In order to expedite prosecution, in accord with the above amendment, the amended claims are directed to treatment of portal hypertension and its bleeding complications.

Claims 1-4 and 8-12 are rejected under 35 U.S.C. §103(a) over Garcia et al. ("Garcia") in view of Garcia-Tsao, and further in view of Niazi et al. (US 6338862; "Niazi"). Applicant respectfully submits that the Examiner's reasoning is based on a fundamental misconception of the reference of Garcia.

In particular, Applicant submits that the Examiner has a total misunderstanding with respect to the substantial differences between "general (systemic) hypertension" on the one hand, and "portal hypertension" specifically on the other hand in connection with the present invention. These expressions define entirely different conditions and disease states. That is, reduced systemic hypertension does not mean reduced portal hypertension, and the difference between both diseases/conditions is particularly striking, for example, in the severe case of human cirrhosis patients (which is the typical cause of portal hypertension as explained in the present application).

It is important not to confuse the meanings and expressions of "systemic pressure", "mean arterial pressure (MAP)" or "systemic hypertension" (or "severe hypertension") as opposed to "portal pressure" or "portal hypertension".

The Examiner's misunderstanding is apparent, e.g., from the following passage at the bottom of page 5 of the Office Action:

> Applicant argues that the 10 mg/kg dosage of sildenafil resulted in an unsafe drop in systemic blood pressure

but, then, the Examiner goes on to state the following consequence "a decrease in systemic blood pressure may also be desired in a patient with severe hypertension." The Examiner is entirely wrong in this position.

In view of these flawed statements of the Examiner, Applicant will explain the background regarding portal hypertension and systemic hypertension, and the information derivable from Garcia by one skilled in the art in more detail. First, it is well understood by those skilled in the art that the mechanisms and circumstances in the case of portal hypertension differ from those found in the case of general (systemic) hypertension, in particular, in cases of liver cirrhosis or associated severe disease conditions such as bleeding complications and the like.

Mechanisms for the generation of portal hypertension are explained in detail, e.g., in publications of D.A. Langer et al., ***J. Hepatol.*** 2006, 44, pp. 209-216 and W. Laleman et al., ***Liver International*** 2005, 25, pp. 1079-1090. In portal hypertension, vaso***constriction***-effective substances predominate **within the liver**, leading to a constriction of the liver sinusoids (i.e. the smallest blood vessels within a liver). In addition, intra-hepatic resistivity is increased, which is typical for the event of liver cirrhosis.

To the contrary, the situation of blood flow **in the periphery**, i.e., through the gastro-intestinal system, is fundamental different; there, vaso***dilatory*** substances predominate.

Shortly circumscribing the situation of liver cirrhosis and the potential risk of getting portal hypertension: there is ***insufficient*** NO (nitric oxide) inside the liver, whereas an ***excess*** of NO occurs **outside** the liver. As a consequence, the event of liver cirrhosis and the situation of

potential high risk of portal hypertension (which is relevant here) are characterized by a pre-existing (i.e. before a therapeutic treatment is considered) **low systemic blood pressure** and a reduced peripheral vascular resistivity. See passages highlighted in the paper of Laleman.

M. Henneberg et al., *Gut* 2008, 57, pp. 1300-1314, further explain the situation of peripheral vasodilatation in the event of portal hypertension. Again, the discrepancy between vasoconstriction within the liver and vasodilatation in the periphery (outside the liver) is described (see the introductory portion). It is further explained that, in the case of portal hypertension, there is a **reduced systolic (i.e., systemic) blood pressure** (increased heart frequency), and a further reduction of systolic (systemic) blood pressure would dramatically worsen the situation (associated with a worsening of the function of kidneys and potentially with renal failure, and worsening of the lungs, the brain function, etc.).

Consequences on the circulation in the event of liver cirrhosis (and thus the potential risk of portal hypertension) is further summarized by J. Bosch in *J. Clin. Gastroenterol.* 2007, 41 Suppl. 3, S247-S253 from a standpoint considering both clinical and animal-experimental results. Consistently therewith, M.H. Tsai, *J. Clin. Gastroenterol.* 2007, 41 Suppl. 3, 5266-5271, and R. Wiest, *J. Clin. Gastroenterol.* 2007, 41 Suppl. 3, 5272-5287, suggest the **inverse** correlation between **vasoconstriction of the liver sinusoids and dilatation of the blood vessels in the periphery outside the liver.** Increased blood pressure within the liver is anti-correlated with a **reduced systemic** blood pressure.

A review of A.J. Sanyal et al. in *Gastroenterology* 2008, 134, pp. 1715-1728 again summarizes: (further) reduction of systemic blood pressure can be **deleterious** in the event of liver cirrhosis (i.e. a condition with high risk of portal hypertension), with a tendency towards generation of hepato-renal syndrome or hepatic encephalopathy.

Clearly, the real situation is contrary to what the Examiner apparently has assumed. Patients having liver cirrhosis (i.e. causing portal hypertension) need to **regain** normal circulation from reduced **to normal systemic** (systolic) **blood pressure.**

Such critical situations and mechanisms are specifically described for example, e.g. by Ruiz-del Arbol et al. in *Hepatology* 2005, 42, pp. 439-447. Reduced systolic blood pressure associated with the condition of liver cirrhosis (i.e. with likelihood of portal hypertension) a high risk of renal failure.

Applicant also draws the Examiner's attention to the statements in R.J. Groszmann et al. in *N. Engl J. Med.* 2005, 353, pp. 2254-2261. The **mean arterial pressure (MAP)**, which is an indication for systemic (systolic) blood pressure, is **at a low level** of 94 mm Hg in patients having early stage **liver cirrhosis**.

From all of the above explanations supported by sound scientific knowledge, Applicant respectfully submits that the following is proven:

- Systolic blood pressure or, as indicated by Garcia, MAP, must **not** be equated with and, in fact, **does** not correspond to portal pressure (PVP).
- Even more, the consequences on vascular pressure in patients having liver cirrhosis and, thus, having a high risk of generating portal hypertension, is characterized by a **contrary/antipodal** relationship: the *systemic* pressure (MAP) is *hazardously **low***, whereas the *portal hypertension* (associated with a constriction of liver sinusoids and, thus, a marked reduction of blood flow through the liver) is *hazardously **increased***!
- Extrapolating the information in Garcia about a 50% reduction of MAP (and still at a lower reduction) to situations of portal hypertension, i.e., patients having liver cirrhosis and, thus, already suffering from a low MAP, would be absolutely deleterious and potentially lethal for humans.

Therefore, it should be appreciated from the above discussion and enclosed scientific publications that the Examiner's assumptions and interpretation of the results described by Garcia are erronious. Garcia teaches that, when using moderate doses of silenafil (0.1 or 1 mg/kg i.v.), given to rats, **no effect in PVP is seen,** whereas when using extremely high **intravenous** doses given to rats, a dramatic 50% decrease in MAP was observed. This diminished MAP **persisted**, which means that the animals were sentenced to death.

The information provided by Garcia, therefore, absolutely are **contra**indicative - in other words **an antithesis** - to a concept of **treating portal hypertension** in patients suffering from liver cirrhosis and *portal* hypertension. In this context, it also should be appreciated that Garcia is not only contraindicative to, but, also does not even attempt to, treat portal hypertension. Rather, systemic and portal haemodynamic effects of usual erectile dysfunction treatments by sildenafil are studied by Garcia. It should be appreciated, further, that the data reported by Garcia are contradictory to the data reported by others in the field, notably by Celle et al. in *Digestive Disease Week* 2003, Abstract No. S1553 (see the discussion in the present specification in the second paragraph on page 5).

Therefore, it is noted that the surprising effects described and demonstrated in the present application (see, e.g., page 4, 5th paragraph to page 8, 3rd paragraph and the results demonstrated for humans in the examples and depicted in the Table as well as the post-filed experimental evidence) would never have been expected by the skilled practitioner from, nor could be derived by the skilled practitioner from, the data reported by Garcia for rats.

It is respectfully submitted that the presently claimed subject-matter clearly would not have been obvious to one of ordinary skill in the art in view of the silenafil data of Garcia. This non-obviousness applies even more to the PDE5 inhibitor of Vardenafil.

Neither Niazi nor Garcia-Tsao make up for the deficiencies of Garcia. None of Garcia, Garcia-Tsao or Niazi, nor any combination thereof teach or suggest any method for the treatment of portal hypertension and the associated conditions, as set forth and claimed in the present application.

It is respectfully submitted that the presently claimed invention would not have been obvious to one of ordinary skill in the art in view of any combination of Garcia, Garcia-Tsao and Niazi.

In view of the discussion above, Applicant respectfully submits that the pending application is in condition for allowance. An early reconsideration and notice of allowance are earnestly solicited.

If, after consideration of the above discussion with enclosed scientic evidence, the Examiner concludes that the present application is not in condition for allowance, Applicant requests that the Examiner call the undersigned attorney to discuss this case so that Applicant can gain further understanding of the Examiner's reasons.

If for any reason a fee is required, a fee paid is inadequate or credit is owed for any excess fee paid, the Commissioner is hereby authorized and requested to charge Deposit Account No. **04-1105**.

Dated: October 6, 2008

Respectfully submitted,

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Review

Nitric oxide and portal hypertension: Interface of vasoreactivity and angiogenesis

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1. Portal hypertension and nitric oxide

1.1. Pathophysiology of portal hypertension

Portal pressure is described mathematically as a function of flow and resistance across the hepatic vasculature, otherwise known as Ohm's law: (*pressure = flow × resistance*) [1]. In cirrhosis, increased intrahepatic resistance results from both intrahepatic vasoconstriction and surrounding mechanical factors including collagen deposition and regenerative nodules. Although recent studies are demonstrating a dynamic component to mechanical factors [2], these are not currently a reliable target for portal pressure reduction. Intrahepatic vasoconstriction is more reversible and probably contributes upwards to 25% of increased resistance [3], however, some recent experimental studies suggest this may be an underestimation of the full effect of intrahepatic vasoconstriction on portal pressure elevation [4].

Within the splanchnic and systemic circulation there is increased cardiac output and hyperdynamic circulation that contributes to increased flow into the portal circulation thereby perpetuating portal hypertension. Vascular remodeling and angiogenesis occurs at the portosystemic interface in attempt to relieve the resulting excess portal pressure and allow decompression by redistributing flow through esophageal and hemorrhoidal collaterals. Unfortunately, the resulting generation of varices is a major contributor to morbidity and mortality.

Nitric oxide (NO) is a central mediator of these vasoreactive and angiogenic abnormalities and is the focus of this review. For more detailed discussion of portal hemodynamics, clinical manifestations, and other non-NO based mechanisms, see other reviews [1,5–7].

1.2. Biology of NO

NO is a hydrophobic gas that freely diffuses across cell membranes and can have autocrine and paracrine effects up to 100 μm in range. It is short-lived with a half-life of perhaps 3–5 s and is rapidly absorbed by heme-containing proteins in the surrounding circulation [8]. NO is generated by the enzyme nitric oxide synthase (NOS) [9]. There are three known isoforms: endothelial, inducible, and neuronal NOS (eNOS, iNOS, nNOS, respectively) [10], each of which generates NO as a byproduct of the conversion of arginine to citrulline [9]. In the quiescent liver, eNOS is the predominant isoform constitutively expressed by sinusoidal endothelial cells and involved in maintaining sinusoidal tone and flow (Fig. 1) [6,11]. NO generated from sinusoidal endothelial cells acts on adjacent hepatic stellate cells at least in part through activation of soluble guanylyl cyclase signaling and culminating in stellate cell relaxation (Fig. 1). This sinusoidal paracrine pathway appears to contribute importantly to hepatic vascular resistance [5–7,12]. Regulation of eNOS is complex and occurs at multiple levels including transcription, mRNA stability and post-translational modifications (Fig. 1) [6]. This is reviewed in detail elsewhere [13,14]. Both iNOS and nNOS have also been implicated in specific aspects of portal hypertension and their biology is equally complex as reviewed elsewhere [15,16].

2. NO deficiency and increased intrahepatic resistance

2.1. Sinusoidal endothelial cells

In the cirrhotic liver, there are multiple derangements in eNOS-derived NO generation that contribute to

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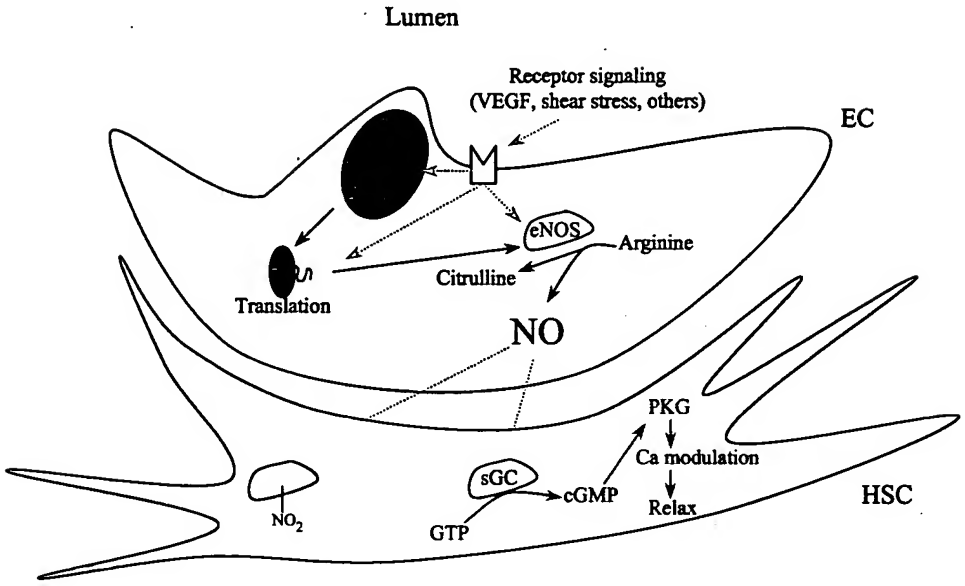

Fig. 1. Nitric oxide regulation within liver sinusoids. Extracellular signals modulate eNOS expression and activity at multiple levels including gene transcription, mRNA stabilization, and post-translational modification (phosphorylation, protein–protein interactions). NO is generated as a by-product from the conversion of arginine to citrulline by eNOS. NO exerts paracrine effects on adjacent hepatic stellate cells through cGMP dependent and independent (protein nitrosylation and peroxynitrite formation) pathways. cGMP signaling cascades through PKG regulate intracellular calcium concentrations leading to HSC relaxation. EC, endothelial cell; HSC, hepatic stellate cell; eNOS, endothelial nitric oxide synthase; sGC, soluble guanylate cyclase; PKG, protein kinase G.

impaired sinusoidal relaxation and increased intrahepatic resistance (Fig. 2) [11,17,18]. In cirrhotic rats there is decreased total NO production in whole liver lysates and isolated endothelial cells [11,18]. Although eNOS protein levels appear to be unchanged, sinusoidal endothelial cells from cirrhotic livers display a prominent increase in the inhibitory protein caveolin binding to eNOS with concomitant decreased calmodulin binding which may contribute to NOS dysfunction [19,20]. Another important factor appears to be the impaired phosphorylation and activation of eNOS by Akt [1]. In cirrhotic rats, there was significantly less phosphorylated eNOS in liver. When rats were transduced with adenovirus containing a constitutively active form of Akt, eNOS activity was increased and portal pressures reduced to normal values [4] (Fig. 2). Thus, multiple molecular defects likely contribute to a significant deficiency in hepatic NO production in cirrhosis.

2.2. Hepatic stellate cells

Not only is there diminished NO production by sinusoidal endothelial cells, but hepatic stellate cells also demonstrate resistance to NO mediated relaxation (Fig. 2)

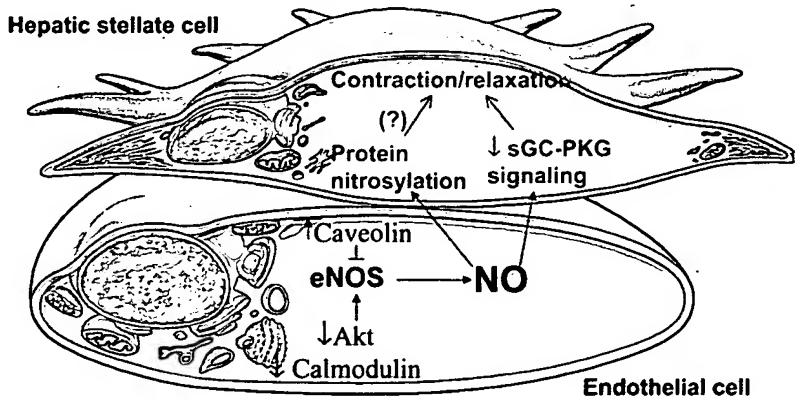

Fig. 2. Intrahepatic NO signaling defects in portal hypertension. NO bioavailability is decreased within liver sinusoids. NO production from sinusoidal endothelial cells is diminished through several mechanisms. Protein levels of eNOS are unchanged but activity is inhibited due to interaction with caveolin, decreased Akt phosphorylation and decreased calmodulin binding. In addition, HSC display blunted relaxation responses to NO that may be, in part, due to decreased cGMP pathway signaling and protein nitrosylation. eNOS, endothelial nitric oxide synthase; sGC, soluble guanylate cyclase; PKG, protein kinase G.

[17]. Rat models of experimental cirrhosis and portal hypertension show decreased vasorelaxation in response to NO donors through a blunted NO response in hepatic stellate cells [17]. Recent published and preliminary studies revealed defects in the guanylate cyclase signaling pathway that may contribute to impaired NO relaxation in activated stellate cells [21–24]. Mechanisms of hepatic stellate cell resistance to NO have important clinical implications as many currently designed therapeutic approaches require optimal vasodilatory effects of NO donors.

2.3. NO for therapy of increased intrahepatic resistance

Animal experiments have demonstrated that activation of hepatic eNOS can improve portal hemodynamics in cirrhotic rats [4,25]. In carbon tetrachloride cirrhotic rats, adenoviral transduction with a constitutively active form of Akt, an upstream kinase activator of eNOS, promoted increased eNOS phosphorylation, cyclic GMP levels and reduction in portal pressures [4]. In addition, adenoviral delivery of nNOS similarly promoted improvement in portal hemodynamics in portal hypertensive rats [25]. Conversely, adenoviral delivery of a constitutively active form of eNOS did not show significant improvements in portal pressure in response to vasoconstrictors in a bile duct ligation model of portal hypertension [21], (although a wild-type construct was effective in a carbon tetrachloride model [26]). Another study evaluated the use of simvastatin as an eNOS activator [33]. Statins such as simvastatin, promote Akt phosphorylation and caveolin-eNOS dissociation, leading to increased NO production [34]. Patients who received simvastatin demonstrated increased hepatic venous NO products and decreased hepatic vascular resistance without untoward systemic vascular effects [33]. These studies suggest that eNOS activity is specifically inhibited within the cirrhotic milieu but that activity can be increased by overexpression of upstream activators or an alternative NOS isoform.

Clinical trials in humans support the role of nitrates to reduce risk of variceal hemorrhage, when used in combination with beta-blockers [27,28]. However, nitrates alone may have a detrimental effect on mortality in cirrhotic patients, with exacerbation of the hyperdynamic circulation and poor tolerance [29]. To overcome the nonspecific nature of systemic nitrates, some studies have focused on hepatic specific NO donors. NCX-1000 is a NO-releasing derivative of ursodeoxycholic acid. In carbon tetrachloride and bile-duct ligated cirrhotic rats, treatment with NCX-1000 resulted in decreased hepatic vasculature resistance, increased cyclic GMP levels, and decreased vasoconstrictor responses without affecting systemic hemodynamics [30,31]. V-PYRRO-NO, another liver specific NO donor, was also shown to protect from endothelial damage in a rat model of sinusoidal obstruction syndrome [32].

3. Excess NO generation in the splanchnic circulation

In contrast to diminished intrahepatic bioavailability of NO, the splanchnic (and systemic) circulation experiences a relative excess in regional NO generation [7]. This increased production is largely endothelium and eNOS dependent [35–37]. However, iNOS and nNOS have been implicated as well and studies in NOS isoform gene deletion mice have not fully clarified the matter [38–41]. There are several mechanisms by which eNOS activation in splanchnic endothelium may occur (Fig. 3). Initial portal hypertension from increased intrahepatic resistance promotes increased shear stress and/or other mechanical stress within the mesenteric circulation [7]. This stress may promote increased eNOS activity by increased transcription, eNOS phosphorylation by Akt, binding to heat shock protein 90, and increased calcium/calmodulin binding with increased intracellular calcium concentrations (Fig. 3) [42–44]. Bacterial translocation in cirrhotics may also contribute to increased NO production, through increased generation of tetrahydrobiopterin (BH4) or AKT phosphorylation [43,45,46]. More recent studies suggest that vascular endothelial growth factor (VEGF) activation of eNOS may be a primary factor in the initial eNOS activation process as well [47]. Thus, NO production in the splanchnic and systemic circulation is increased through multiple mechanisms and contributes to the decreased systemic vascular resistance and resultant hyperdynamic circulation.

3.1. NO inhibition therapies

Based on the above underlying mechanisms, several experimental treatment approaches have been evaluated. In fact, many of the currently established therapies for portal hypertension also act, in part by regulating NO. For example, in portal vein stenosed rats, beta-blockers were shown to decrease shear stress mediated eNOS activation and prevent aortic hyporesponsiveness to vasoconstrictors in addition to reducing NO dependent collateral blood flow [48,49]. Additionally, the vasoconstrictor terlipressin was shown to inhibit aortic NOS expression and subsequent NO production in rats [39].

In terms of novel targets for future therapies, endogenous cannabinoids may also promote splanchnic vasodilation although the role of eNOS in cannabinoid vasodilation is controversial [50,51]. Regardless, in cirrhotic rats, systemic hypotension was reversed with a cannabinoid receptor antagonist [50,51]. Antibiotics may also be useful. To address production of inflammatory mediators from bacterial translocation, alcoholic cirrhotic patients were treated with norfloxacin for intestinal decontamination; these patients showed decreased serum endotoxin levels, increased systemic vascular resistance, increased mean arterial pressure and trends for decreased cardiac output and hepatic wedge pressure [52]. This effect was thought to be

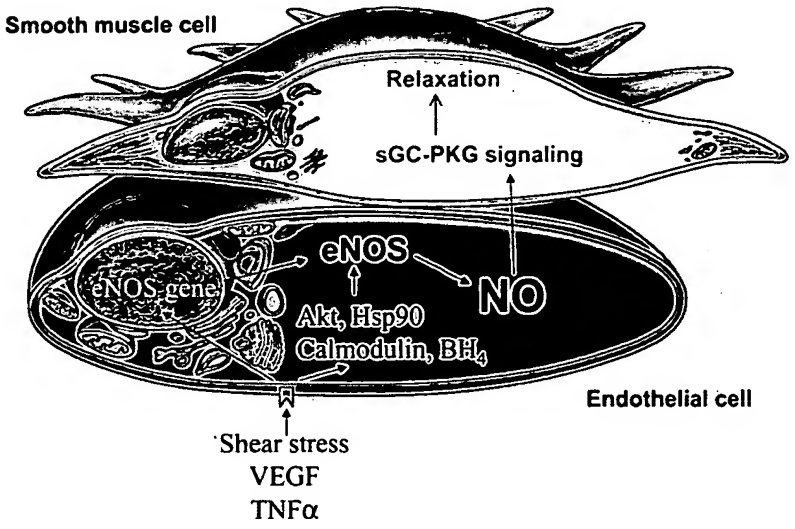

Fig. 3. Extrahepatic (splanchnic) NO signaling defects in portal hypertension. Increased NO generated by splanchnic and systemic endothelial cells promotes surrounding smooth muscle relaxation that results in decreased systemic vascular resistance. eNOS is stimulated through several pathways. Extracellular stimulatory signals include shear stress, VEGF, and TNFα possibly from bacterial translocation and increased endotoxin levels. These signals promote increased eNOS transcription. Post-translational modifications that promote increased enzyme activity include Akt mediated phosphorylation of eNOS and increased eNOS binding of activators/cofactors (calmodulin, tetrahydrobiopterin, and Hsp90). BH_4, tetrahydrobiopterin; VEGF, vascular endothelium growth factor; eNOS, endothelial nitric oxide synthase; sGC, soluble guanylate cyclase; PKG, protein kinase G.

mediated through decreased TNFα levels [43,45,46]. The role of TNF has been further evidenced by a study in patients with alcoholic hepatitis and portal hypertension who received the anti-TNFα antibody, infliximab [53]. These patients showed improvements in hemodynamics including hepatic venous pressure gradient and cardiac output and circulating levels of inflammatory cytokines [53]. Based on current understanding, treatments that promote systemic inhibition of NO synthesis and function hold future promise, particularly therapies targeting VEGF, TNF, cannabinoids, and bacterial products.

4. Collateral circulation

The development of the portosystemic collateral circulation is an important complication of portal hypertension. Esophageal and hemorrhoidal collateral vessels develop from increased portal pressure as a compensatory response to decompress the portal circulation, but unfortunately contributes to significant morbidity and mortality. NO may play a critical role here by promoting collateral flow through dilation of pre-existing vessels, as well as through promoting vascular remodeling and angiogenesis [47, 54–58].

Vasodilation of pre-existing collateral vessels is a NO dependent process likely mediated through mechanical forces such as shear-stress and growth factors such as VEGF. In portal vein ligated rats, portosystemic shunting was inhibited by NOS inhibitors [59–61]. Current therapy for varices with non-selective beta-blockers is directed in part at this vasodilation. Indeed, azygos blood flow as a measure of porto-systemic shunting was significantly reduced in cirrhotic patients after receiving propranolol [62] and thus NO inhibition mediates some of the protective effects from beta-blockers.

Vascular remodeling is a longer-term adaptive response to chronic changes in blood flow. Remodeling allows sustained increases in flow capacity and vessel diameter. In vessels of cirrhotic rats, wall thickness was decreased, an effect also reversed with NOS inhibition [63]. Furthermore, in eNOS knockout mice there was decreased vessel remodeling after ischemic injury with an increase in wall thickness with hyperplastic response of surrounding smooth muscle cells [64]. Vascular remodeling also occurs in the cirrhotic hepatic circulation as NO influences endothelial cell and hepatic stellate cell migration and proliferation, steps that are key to vascular remodeling [23,65].

Recent evidence points to a predominant role for angiogenesis in collateral vessel formation [47]. Angiogenesis occurs through the proliferation of in situ endothelial and smooth muscle cells in addition to vasculogenesis (Fig. 4) [66,67]. Vasculogenesis refers to recruitment of endothelial progenitor cells [68] for the synthesis of vessels de novo. Portal hypertensive rats were shown to promote increased angiogenesis in explanted collagen rings; this effect was prevented by NOS inhibition [55,56]. eNOS is also required for optimal mobilization of endothelial cell precursors from the bone marrow (Fig. 4) [57]. Bone marrow derived endothelial progenitors were shown to be a major contributor to the regenerating endothelium in mice after hepatectomy although their role in cirrhotic remodeling is less established [69]. Angiogenesis is highly dependent on VEGF as this growth factor exerts pleiotropic

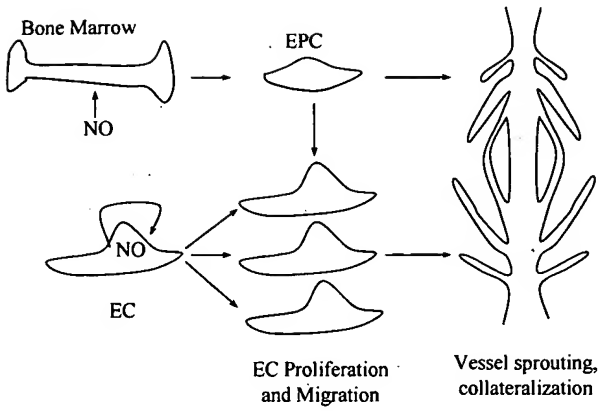

Fig. 4. NO promotes angiogenesis and collateralization. VEGF may act through stimulation of eNOS derived NO which then promotes local endothelial cell proliferation and migration leading to new vessel formation. NO derived from eNOS is also necessary for bone marrow mobilization of endothelial progenitor cells which home to areas of neovascularization contributing to new vessel formation and stimulating surrounding endothelium through paracrine mediators. EPC, endothelial progenitor cell; EC, endothelial cell; eNOS, endothelial nitric oxide synthase.

effects to promote new vessel formation [54]. Indeed, VEGF promotes vasodilation, vascular remodeling and angiogenesis, in part, through NO dependent mechanisms, although NO independent effects of VEGF are important and equally well established [70]. In animal models, neutralizing antibody to VEGF receptor 2 significantly inhibited portosystemic shunting, further supporting the importance of VEGF and NO for increased collateralization [47]. Targeting of VEGF and progenitor cells at sites of systemic collaterals is certainly a promising therapeutic approach for portal hypertension and is an area of active investigation. Conversely, perhaps stimulating angiogenesis and collateralization at appropriate sites (i.e. intrahepatic shunts, splenorenal collaterals) could enhance portal pressure decompression, in the absence of major morbidity.

5. Other organ systems affected by NO signaling derangements

5.1. NO in hepatic encephalopathy

The molecular mechanisms of hepatic encephalopathy are complex and under active investigation. However, there is evidence that NO may contribute. Experiments using brain lysates from porto-caval anastamosed rats and cirrhotic human samples have helped delineate potential NO dependent pathways modulated by high ammonia levels [71–75]. It has been postulated that ammonia may promote nNOS derived NO generation which increases cerebral blood flow and promotes glutamine synthetase nitration [71–73,75]. Thus, NO generated by nNOS may be important in pathways mediating hepatic encephalopathy and may represent a novel therapeutic target.

5.2. NO in hepatopulmonary syndrome

Hepatopulmonary syndrome occurs in up to 15% of cirrhotic patients [76]. This syndrome is characterized by pulmonary vascular dilations that result in shunting and subsequent hypoxemia. Experiments using bile-duct ligated rats as an animal model of cirrhosis and hepatopulmonary syndrome indicate a vasodilatory role for the classic vasoconstrictor endothelin-1. Endothelin-1 can act on endothelin-B receptors within the pulmonary vasculature and cause increased eNOS activity and NO production ultimately leading to pulmonary vessel dilatation [76]. This increased eNOS activity and vasodilation is reversed by NOS inhibitors [77]. Administration of methylene blue, a NO and soluble guanylyl cyclase inhibitor, to cirrhotic patients with severe hepatopulmonary syndrome resulted in improved oxygenation and pulmonary hemodynamics [78]. Additionally, pentoxifylline, which is thought to inhibit TNF and macrophage derived NO, may be a promising direction of treatment as well [79].

In contrast to hepatopulmonary syndrome, portopulmonary hypertension is a clinical condition where vasoconstriction, mediated by endothelin and smooth muscle proliferation, result in the setting of a hyperdynamic circulation [80]. Enhanced local NO production or targeted administration may be a useful clinical adjunct but has not been examined in great detail, in part due to lack of good experimental models. The phosphodiesterase inhibitor, sildenafil, which increases NO dependent signaling, may warrant further evaluation owing to its potential benefit in primary pulmonary hypertension [81].

5.3. NO in cirrhotic cardiomyopathy

NO is also involved in the pathogenesis of cirrhotic cardiomyopathy [82]. The ability to distinguish cardiomyopathy in the setting of cirrhosis and concomitant hyperdynamic circulation can be difficult. The hemodynamics of patients with advanced cirrhosis were evaluated, and it was discovered that there was a significant decrease in stroke volume related to decreased cardiac contractility or diastolic dysfunction [83]. This diminished contractility may contribute to the decreased effective circulating volume and ensuing complications of cirrhosis such as spontaneous bacterial peritonitis and hepatorenal syndrome [84,85]. Animal studies suggest the pathogenesis of cirrhotic cardiomyopathy is mediated by iNOS generation of NO that inhibits contractility; this effect was reversed with NOS inhibitors and exacerbated by NO donors [86]. The role of cirrhotic cardiomyopathy in human portal hypertension continues to be an area of active investigation and may be particularly relevant in the peri-transplant setting.

5.4. NO in portal hypertensive gastropathy

Portal gastropathy is common in cirrhosis and contributes to gastrointestinal bleeding. In animal models of portal hypertension with gastropathy, TNFα and eNOS levels are elevated [45]. In experiments delineating signaling pathways, it was shown that TNFα promotes eNOS activation by Akt phosphorylation [45]. This effect was abrogated by administration of anti-TNFα neutralizing antibody [45,87]. However, the overall effect of NO on the mucosal barrier function remains controversial.

5.5. NO in hepatorenal syndrome

The role of NO in hepatorenal syndrome is complex owing to the combination of local and regional hemodynamic alterations that contribute to this syndrome including systemic, hepatic, cardiac, and renal alterations [84,88]. Medical therapies are currently directed at the NO mediated hyperdynamic circulatory changes [88], although ultimately, liver transplant is the only current therapy for hepatorenal syndrome with long-term reversal.

6. Summary

Altered NO signaling plays a central role in the pathogenesis of portal hypertension and many of its complications. Intrahepatic NO synthesis and downstream signaling are inhibited at multiple levels promoting vasoconstriction and increased intrahepatic resistance. Within the splanchnic and systemic circulation there is excess NO production resulting in vasodilation, decreased systemic vascular resistance and increased portal inflow. The portal circulation attempts to decompress through collateral vessels where NO is involved in promoting vasodilation, vascular remodeling and angiogenesis. Alterations in NO synthesis and function also contribute to other complications of portal hypertension including hepatic encephalopathy, hepatopulmonary syndrome, cirrhotic cardiomyopathy, portal hypertensive gastropathy and the hepatorenal syndrome. Although advances in organ-specific delivery are needed, targeted modulation of NO pathways holds great promise for therapeutics in portal hypertension.

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Mechanisms of extrahepatic vasodilation in portal hypertension

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ABSTRACT

In liver cirrhosis, abnormal persistent extrahepatic vasodilation leads to hyperdynamic circulatory dysfunction which essentially contributes to portal hypertension. Since portal hypertension is a major factor in the development of complications in cirrhosis, the mechanisms underlying this vasodilation are of paramount interest. Extensive studies performed in cirrhotic patients and animals revealed that this vasodilation is associated on the one hand with enhanced formation of vasodilators, and on the other hand with vascular hyporesponsiveness to vasoconstrictors. The latter phenomenon has been termed "vascular hypocontractility". It is caused by a combination of different mechanisms and factors described in this review.

In cirrhosis of the liver, persistent vasodilation of extrahepatic vessels leads to increased portal venous inflow and contributes to portal hypertension (fig 1). Portal hypertension is determined by the degree of hepatic outflow resistance and the extent of inflow into the portal venous system from the splanchnic bed, since—according to Ohm's law applied to the vascular system—portal pressure is the product of portal venous blood flow and resistance to flow.

However, vasodilation in cirrhosis is not restricted to splanchnic vessels. It also affects the central venous and peripheral circulation.[1–3] Although an intrahepatic increase in resistance is the dominant factor for the development of portal hypertension, extrahepatic vascular changes, apart from their contribution to portal hypertension, are of major relevance for dysfunction of other organs apart from the liver. Thus, vasodilation in the splanchnic bed may lead to sequestration of the blood pool with subsequent central vascular underfilling and activation of baroreceptors, causing increased levels of vasoconstrictors.[4–9] These in turn are a driving force for kidney dysfunction.[5] Pulmonary impairment and unbalanced cerebral perfusion as well as cardiac malcontraction are also believed to result from functional alterations of the vascular system outside the liver.[1 4–10]

To what extent these alterations are already present in the stage of compensated cirrhosis or how far they mirror decompensated liver function remains unclear. While it has also been shown that the extrasplanchnic vasculature in some organs and territories—in addition to the kidney—may even be hypercontractile (eg, in the brain or limbs),[1] the majority of the vessels are dilated despite systemic activation of vasoconstrictors.[4–9] This typically results in clinical symptoms and signs in patients with liver cirrhosis, such as relatively low blood pressure together with an increased heart rate, warm skin, cutaneous spider angioma or capillary pulsation of the digits.

Animals with experimentally induced portal hypertension are characterised by splanchnic vasodilation, increased splanchnic flow, as well as diminished systemic vascular resistance and hypotension. The decrease in systemic and splanchnic vascular resistance is mainly due to the contribution of microvessels with diameters of $\leqslant 150$ µm. However, as it has been shown that they exhibit a similar dysregulation, investigators have used isolated aortas and mesenteric arteries to study the intracellular mechanisms of vasodilation in animal models.[11]

Vascular functional alterations are associated not only with an augmented formation and action of vasodilators, such as nitric oxide (NO), and with impaired responsiveness to vasoconstrictors (ie, disturbed vasomotor regulation), but also with vascular remodelling and increased angiogenesis.[12] The latter phenomena are beyond the scope of this review.

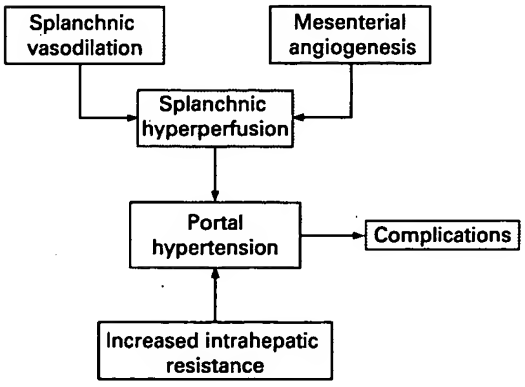

Figure 1 Role of extrahepatic vasodilation in portal hypertension and its complications in cirrhosis of the liver. Arterial vasodilation is especially prominent within the splanchnic vascular bed, but not restricted to that. Cirrhotic patients and animals with experimentally induced cirrhosis are characterised by arterial hypotension and decreased systemic and splanchnic vascular resistance. Persistent mesenteric vasodilation causes increased portal inflow, which contributes essentially to the development of portal hypertension. In parallel, portal hypertension is triggered by an impaired portal outflow, which is related to an increased intrahepatic vascular tone.

AIM

It is the aim of the present review to summarise the current knowledge of intracellular mechanisms

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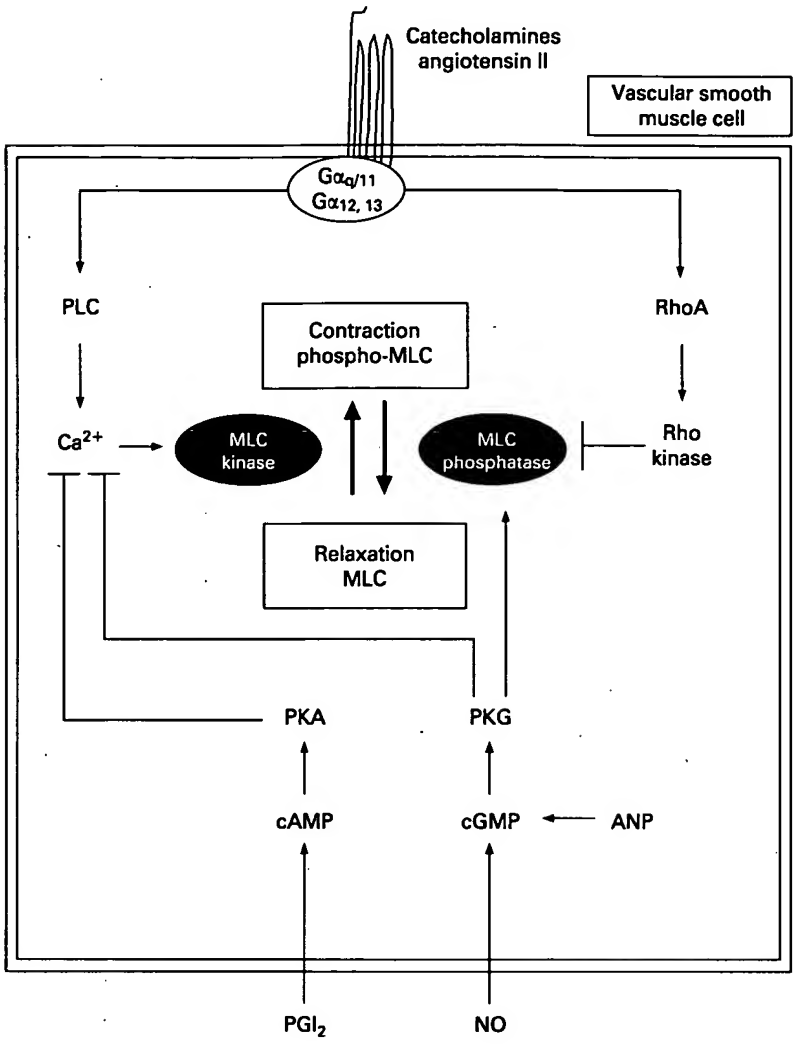

Figure 2 Regulation of vascular tone by vasomotoric signalling pathways. The contractile state of vascular smooth muscle depends on the phosphorylation state of myosin light chains (MLCs), which is determined by the activities of MLC kinase and MLC phosphatase. All pathways leading to contraction or relaxation exert their effects via changes in MLC kinase or MLC phosphatase activity. Binding of vasoconstrictors (noradrenaline, angiotensin II) to their corresponding G-protein-coupled receptors (α_1-adrenoceptors, angiotensin II type 1 receptor) causes concomitant but parallel activation of two contractile signalling pathways. These are the phospholipase C (PLC)/inositol-1,4,5-trisphosphate–Ca^{2+}/calmodulin pathway (left side) and the RhoA/Rho kinase pathway (right side). Activation of these contraction cascades results in activation of MLC kinase and inhibition of MLC phosphatase. Endothelium-dependent vasorelaxation occurs via nitric oxide (NO) and prostaglandins (PGI_2), which are produced in endothelial cells in response to various different mediators (e.g. acetylcholine, bradykinin, vascular endothelial growth factor) and shear stress. Via formation of cyclic nucleotides (cGMP, cAMP) and subsequent activation of their effectors—that is, cGMP- and cAMP-dependent kinase (PKG, PKA), NO and PGI_2 result in relaxation of the adjacent vascular smooth muscle. Endothelium-independent vasorelaxation comes from circulating and nervous mediators, which act directly at vascular smooth muscle cells, but also use cyclic nucleotides for relaxation. ANP, atrial natriuretic protein.

which cause vasodilation in hepatic cirrhosis. We will refer not only to the haemodynamic alterations but particularly also to different pathways in different cells and to general mechanisms of vasoregulation. The review is, to a large extent, based on animal models. Although pathophysiology may vary between different animal models, aetiologies and stages of liver cirrhosis, a better understanding of this altered vasoregulation may open the door for new therapeutic strategies, as already shown by the use of vasoactive treatment for hepatorenal syndrome or portal pressure reduction in the event of acute variceal bleeding.

PHYSIOLOGY OF VASCULAR SMOOTH MUSCLE

Contraction of vascular smooth muscle

The contractile state of vascular smooth muscle (VSM) is determined by the simultaneous action of contractile and relaxing pathways (fig 2). Myosin light chain (MLC) phosphorylation represents the ultimate prerequisite required for contraction of smooth muscle cells and is tightly regulated by MLC kinase and MLC phosphatase.[13–16] All vasomotor pathways exert their effects via changes in the activity of either MLC kinase or MLC phosphatase. Contractile pathways increase MLC phosphorylation via activation of MLC kinase or inhibition of MLC phosphatase. In contrast, pathways leading to vasorelaxation decrease MLC phosphorylation via deactivation of MLC kinase or activation of MLC phosphatase.[17–20]

Contractile pathways in VSM are activated after stimulation of G-protein-coupled receptors (GPCRs) by vasoconstrictors (fig 2). Two of the best characterised and most ubiquitous vasopressor receptors are the α_1-adrenoceptor and the angiotensin II type 1 receptor (AT_1R). Both are coupled to heterotrimeric G-proteins containing $G\alpha_{q/11}$, $G\alpha_{12}$ and $G\alpha_{13}$ subunits. After receptor activation by catecholamines or angiotensin II, these Gα-proteins dissociate from their β/γ-subunits and the receptor, and subsequently activate their downstream effectors—that is, phospholipase Cβ (PLCβ) and the small monomeric GTPase, RhoA.[14–16] PLC and RhoA are already part of two different contraction cascades, which are activated in parallel and lead to an increased MLC phosphorylation state through different mechanisms (fig 2).

Activation of PLCβ leads to the formation of the second messengers IP_3 (inositol-1,4,5-trisphosphate) and DAG (diacylglycerol) by hydrolysis of its substrate, phosphatidylinositol-4,5-bisphosphate (PIP_2) (fig 3). IP_3 induces depolarisation of the VSM cell by opening sarcoplasmatic Ca^{2+} channels. This results in opening of voltage-gated Ca^{2+} channels within the cell membrane, subsequent elevation of cytosolic [Ca^{2+}] and activation of the calmodulin (CaM)-dependent MLC kinase (fig 3). In parallel, DAG activates several isoforms of protein kinase C (PKC), of which some are capable of contributing to vasocontraction independently of [Ca^{2+}]. PKC increases the Ca^{2+} sensitivity of the VSM cell by inhibition of MLC phosphatase, so that changes in MLC phosphorylation may occur even at constant cytosolic Ca^{2+} concentrations. Importantly, this mechanism of Ca^{2+} sensitisation is shared by the RhoA/Rho kinase pathway, which is activated in parallel to the IP_3/DAG pathways (fig 3).[13–16] Thus, in addition to PLCβ, receptor-associated Gα-proteins activate RhoA, which subsequently activates Rho kinase. G-protein-induced RhoA activation is

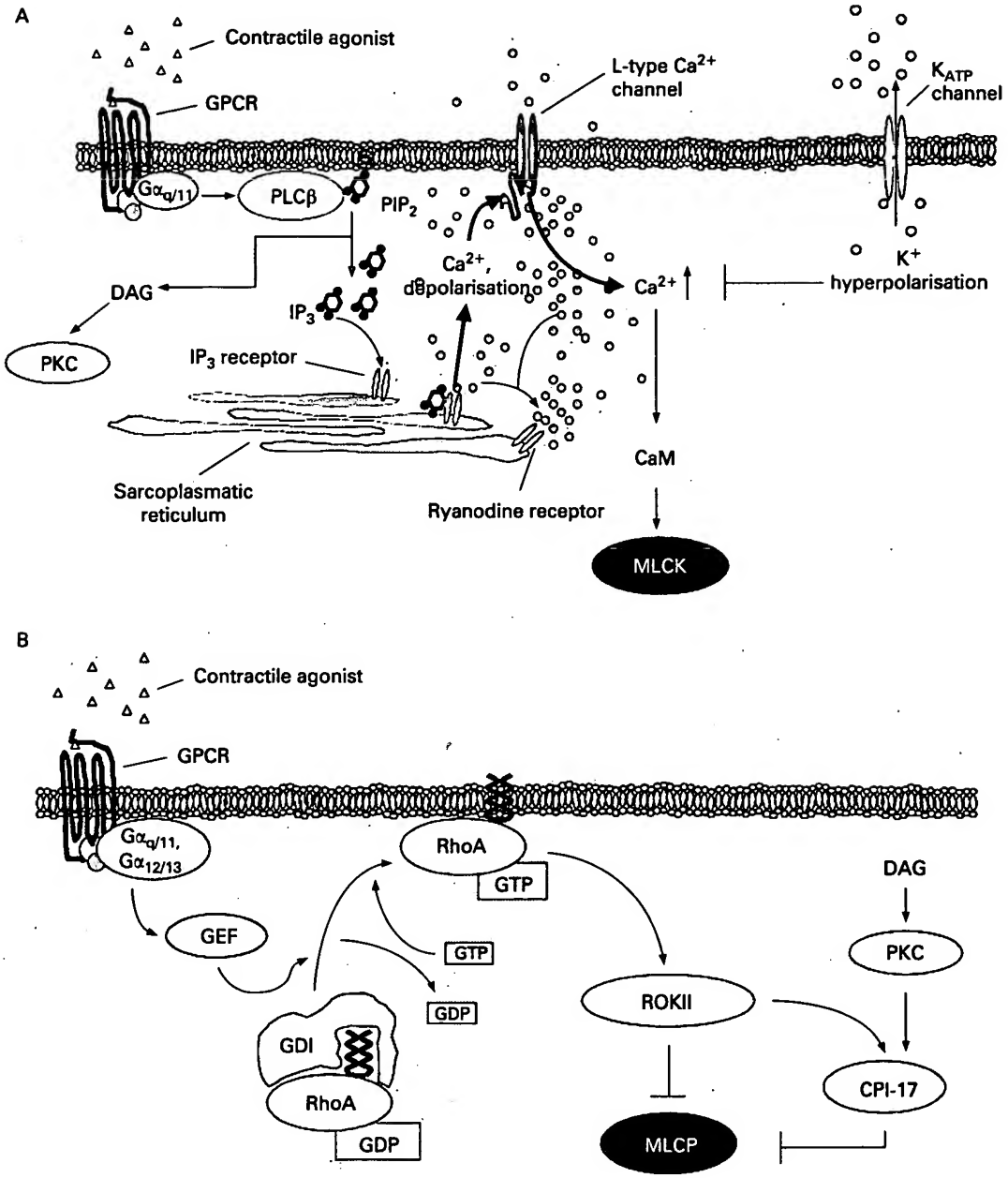

Figure 3 Contractile pathways in vascular smooth muscle. (A) Activation of G-protein-coupled vasoconstrictor receptors (GPCRs; α_1-adrenoceptors, angiotensin II type 1 receptor) leads to activation of receptor-associated heterotrimeric G-proteins. $G\alpha_{q/11}$ and $G\alpha_{12/13}$ subsequently activate their downstream effectors—that is, phospholipase C (PLC) (A) and RhoA (B). Synthesis of the second messenger inositol-1,4,5-trisphosphate (IP_3) by PLCβ causes the opening of IP_3-gated sarcoplasmatic Ca^{2+} channels. This results in depolarisation of the cell, and opening of voltage-dependent Ca^{2+} channels (L-type Ca^{2+} channel) in the cell membrane, followed by an influx of Ca^{2+} and activation of Ca^{2+}/calmodulin (CaM)-dependent myosin light chain kinase (MLCK). Ca^{2+} influx via voltage-dependent Ca^{2+} channels may be prevented by hyperpolarisation due to activation of K_{ATP} or other K^+ channels. (B) In parallel, vasoconstrictor stimulation induces G-protein-mediated activation of the small monomeric GTPase RhoA in vascular smooth muscle. RhoA activation requires an exchange of GDP by GTP at the protein and its translocation to the cell membrane. The GDP/GTP exchange is exerted by receptor/G-protein-controlled nucleotide exchange factors (GEFs), whereas the membrane association of RhoA is regulated by GTPase dissociation inhibitor (GDI), which retains the inactive RhoA in the cytosol. RhoA activates Rho kinase (ROKII), which causes dual inhibition of MLC phosphatase (MLCP): first, Rho kinase inhibits MLCP by phosphorylation of the MLC-binding subunit, MYPT1. Next, Rho kinase phosphorylates the MLCP-inhibiting protein, CPI-17. Phosphorylation of CPI-17 by Rho kinase increases its inhibitory effect towards MLCP 1000- to 2000-fold. Finally, inhibition of MLCP enhances MLC phosphorylation. DAG, diacylglycerol; PKC, protein kinase C.

believed to be mediated by guanosine nucleotide exchange factors (GEFs). However, the exact mechanisms are still under investigation. Rho kinase causes dual inhibition of MLC phosphatase: first, Rho kinase phosphorylates the substrate-binding subunit of MLC phosphatase, MYPT1, at Thr696, and thereby inactivates MLC phosphatase. Secondly, Rho kinase causes inhibition of MLC phosphatase via activation of the PKC-potentiated inhibitor of 17 kDa protein, CPI-17 (fig 3). CPI-17 is phosphorylated by both PKC and Rho kinase at Thr38, which increases the inhibitory effect of CPI-17 towards MLC phosphatase 1000- to 2000-fold. The essential role of Rho kinase in vasocontraction was demonstrated using Rho kinase inhibitors such as Y-27632 and fasudil, which are capable of completely inhibiting contraction of isolated vessels, and inducing sustained decreases in blood pressure in vivo.[21 22]

Signalling cascades leading to vasorelaxation

Vasorelaxation is mediated by the cyclic nucleotides, cGMP and cAMP.[17-20 23 24] Both activate their corresponding effectors, cGMP-dependent protein kinase (PKG) and cAMP-dependent protein kinase (PKA), respectively, which are central mediators of smooth muscle relaxation. These kinases cause vasorelaxation either by interfering with contractile signalling or by activation of MLC phosphatase (fig 2). Synthesis of cGMP or cAMP is initiated by specific cyclic nucleotide cyclases—that is, guanylyl cyclases (GCs) or adenylyl cyclases (ACs). These in turn are activated by various signalling molecules, produced in response to circulating hormonal, nervous or paracrine mediators (fig 2).

One of the best characterised vasodilators is nitric oxide (NO), a small gaseous, diffusible signalling molecule produced by NO synthases (NOSs). Under normal conditions, the main sources of endogenously released NO in vessels are the constitutive NOS isoforms, endothelial NOS (eNOS) expressed in the vascular endothelium, and neuronal NOS (nNOS) found in perivascular non-adrenergic non-cholinergic (NANC) neurons. The third isoform, inducible NOS (iNOS), does not play a role in vasoregulation under normal conditions, and it is mainly expressed in macrophages. After its production within the endothelium or after its release as a neurotransmitter, NO diffuses into adjacent VSM cells where it activates the NO-sensitive GC, resulting in the synthesis of cGMP (fig 2). cGMP subsequently activates PKG, which mediates vasorelaxation.[17-20]

In endothelial cells, NO itself is produced upon activation of eNOS by circulating and paracrine hormones or neurotransmitters, such as acetylcholine, bradykinin, adrenomedullin, endocannabinoids, sphingosine 1-phosphate and others (figs 2 and 4), or shear stress.[25] After binding to their GPCRs on endothelial cells, these hormones activate PLCγ and therewith induce an increase in cytosolic [Ca^{2+}] within the endothelium, which activates Ca^{2+}/CaM-dependent eNOS (figs 2 and 4). In addition to Ca^{2+}-dependent activation, the activity of eNOS underlies a variety of regulatory mechanisms, including phosphorylation, interaction with regulatory proteins and availability of cofactors. Of these, regulation of eNOS by phosphorylation through Akt/phosphatidylinositol 3-kinase (PI3K) has attracted most attention (fig 4). Another source of cGMP within vascular smooth muscle cells is represented by the membrane-associated GCs ("particular GC", pGC) which are coupled to the receptors for natriuretic proteins, including atrial natriuretic protein (ANP).[26]

PKG mediates vasorelaxation via two mechanisms, namely activation of MLC phosphatase, by MYPT1 phosphorylation at Ser850, and a concomitant decrease in cytosolic [Ca^{2+}] (fig 2). The latter occurs by inhibition of IP_3-gated sarcoplasmatic Ca^{2+} channels due to phosphorylation of the inositol receptor-associated protein kinase G-substrate (IRAG), and by closure of voltage-dependent Ca^{2+} channels in the cell membrane, after hyperpolarisation due to PKG-mediated opening of potassium channels (high-conductance big Ca^{2+}-dependent K^+ channels, BK_{Ca} channels) in the cell membrane.

Vasorelaxation also occurs in response to activation of β-adrenoceptors and prostaglandin receptors at VSM cells. Prostaglandins are produced by the endothelium upon stimulation. Via heterotrimeric G-proteins containing $G\alpha_s$-subunits, these

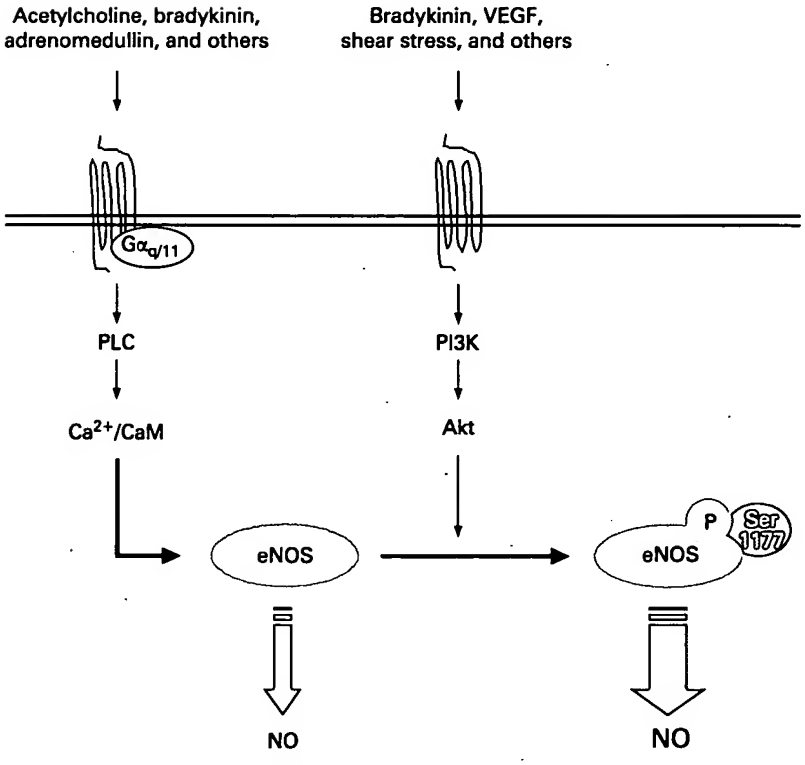

Figure 4 Activation and post-translational regulation of endothelial nitric oxide synthase (eNOS). Apart from its expression level, eNOS activity is determined by the cytosolic Ca^{2+} concentration and its phosphorylation state at different sites. Thus, eNOS is activated by Ca^{2+}/calmodulin (CaM) in response to an elevation of [Ca^{2+}]. Independently, any given eNOS activity is increased by phosphorylation at Ser1177 (according to human eNOS). Several stimuli use both mechanisms simultaneously to mediate eNOS activation. NO, nitric oxide; PI3K, phosphatidylinositol 3-kinase; PLC, phospholipase C; VEGF, vascular endothelial growth facor.

receptors are coupled to ACs: after its activation by receptors, $G\alpha_s$ activates AC (fig 2). Subsequent activation of PKA results in a decrease in cytosolic $[Ca^{2+}]$.[23 24] Thus, the molecular mechanisms involved in PKA-mediated vasorelaxation are limited compared with those used by PKG.

MECHANISMS OF VASCULAR HYPOCONTRACTILITY AND VASODILATION IN CIRRHOSIS

Many studies performed with cirrhotic patients or animals with experimentally induced cirrhosis and portal hypertension were aimed at revealing the mechanisms underlying vasodilation in cirrhosis of the liver. From these studies it has become obvious that vasodilation and vascular hypocontractility in cirrhosis cannot be referred to a single mechanism.

Role of NO for vasodilation

A large body of evidence unmasked vascular overproduction of NO as one of the hallmarks involved in the pathogenesis of vasodilation in cirrhosis. In cirrhotic patients, increased NO production in mesenteric vessels and hepatic arteries is paralleled by elevated systemic levels (plasma, urine) of nitrate/nitrite and cGMP.[27-29]

Similarly, increased NO and cGMP production in hypocontractile vessels (splanchnic vasculature, aorta), as well as increased levels of NO metabolites and cGMP are typical features of rats with secondary biliary cirrhosis induced by bile duct ligation (BDL),[11 30] with micronodular cirrhosis after chronic CCl_4 intoxication,[31 32] or with non-cirrhotic portal hypertension due to partial portal vein ligation (PVL).[33-36]

In all animal models of experimentally induced cirrhosis and portal hypertension, as well as in cirrhotic patients, vasodilation is associated with upregulation of eNOS expression in mesenteric arteries and aortas (table 1). Thus, eNOS upregulation was observed in aortas and splanchnic vessels from BDL,[37 38] CCl_4[39-42] and PVL rats,[43] and in hepatic arteries from cirrhotic patients (own observations). From the animal models (PVL rats) at least it can be derived that eNOS upregulation precedes the hyperdynamic circulatory changes.[44]

Table 1 Factors leading to extrahepatic eNOS hyperactivity during cirrhosis

eNOS overexpression	Bacterial translocation from the gut to mesenteric lymph nodes, endotoxaemia
	Circulating TNFα, monocyte TNFα
	Shear stress
	Circulating eNOS-activating vasodilators
eNOS activation and post-translational regulation	Increased responsiveness to circulating vasodilators→enhanced G-protein and Ca^{2+}/CaM-mediated eNOS activation
	Increased Akt-mediated eNOS Ser1177 phosphorylation
	Upregulation of SK_{Ca} channels in endothelial cells
	Increased BH(4)-availability due to GTP-cyclohydrolase upregulation and bacterial translocation
	Increased interaction of eNOS with Hsp90

BH(4), tetrahydrobiopterin; CaM, calmodulin; eNOS, endothelial nitric oxide synthase; Hsp90, heat shock protein 90; SK_{Ca} channels, small-conductance Ca^{2+}-dependent K^+ channels; TNF, tumour necrosis factor

Surprisingly, iNOS does not appear to be involved in the increased NO production in cirrhosis.[39 43 45 46] Upregulation of iNOS or increased iNOS activity has only been observed during the first days after surgery for BDL or PVL, but not in advanced experimental cirrhosis.[45] One possible explanation for the lack of iNOS induction despite increased lipopolysaccharide (LPS) concentrations is an inhibition of iNOS expression by eNOS-derived NO.[43]

Upregulation of eNOS is essentially triggered by tumour necrosis factor α (TNFα). TNFα production by monocytes and plasma levels of TNFα are elevated in experimental and human cirrhosis and portal hypertension.[47-51] Blunting of TNFα-mediated signalling by TNFα antibodies or inhibition of TNFα production partially normalised eNOS expression and NO production in PVL rats, which was associated with an improvement of haemodynamic dysregulation.[52 53] Similarly, TNFα antibodies reduced eNOS overexpression and Akt activity in aortas from BDL rats.[49]

Another factor participating in eNOS upregulation is increased bacterial translocation from the gut to mesenteric lymph nodes.[48 51] Advanced experimental and human cirrhosis is associated with increased permeability of the gut and with elevated endotoxin concentrations in the plasma and the mesenteric lymph nodes. This has been linked to eNOS upregulation in extrahepatic vessels. It remains to be established to what extent endotoxaemia and increased TNFα levels are part of the same process that contributes to eNOS upregulation, since LPS might cause TNFα production,[54] and treatment of BDL rats with norfloxacin reduces plasma TNFα and eNOS expression.[49] Shear stress was identified as a further important factor triggering eNOS upregulation in portal hypertensive rats.[38 55]

Apart from upregulation of eNOS, eNOS activity is also enhanced by overactivation of signalling pathways resulting in eNOS activation (table 1). In general, eNOS is activated by Ca^{2+}/CaM, and in parallel regulated by phosphorylation at several sites.[56 57] Phosphorylation of eNOS at Ser1197 (according to the rat protein, which is Ser1177 in human eNOS) by Akt/PI3K increases eNOS activity at any Ca^{2+} concentration.[58] Recently, it was demonstrated that development of vasodilation in mesenteric vessels occurs with progressive eNOS phosphorylation at Ser1197. Increased Akt/PI3K activities contribute to eNOS hyperphosphorylation at this site in BDL and PVL rats.[59] The increase in Akt/PI3K activity in hypocontractile vessels results from endotoxaemia due to increased bacterial translocation to mesenteric lymph nodes.[49] Parallel to this, Ca^{2+}-dependent eNOS activity is also increased in aortas from BDL rats. This is related to upregulation of small-conductance Ca^{2+}-dependent K^+ channels (SK_{Ca} channels) in endothelial cells, resulting in an abnormal increase in SK_{Ca} channel-mediated Ca^{2+} influx.[60]

Gut 2008;57:1300–1314. doi:10.1136/gut.2007.144584

Apart from endotoxaemia and TNFα, shear stress was identified as another key factor that leads to eNOS hyperactivity. Elegant studies performed in PVL rats demonstrated that even mild increases in portal pressure are capable of inducing rapid eNOS hyperactivity in mesenteric vessels by a vascular endothelial growth factor (VEGF)-dependent mechanism.[61] Apparently, the development of eNOS hyperactivity starts with Akt-mediated eNOS phosphorylation,[59] which is followed by upregulation of the enzyme later on.[61 62] Since vasoconstriction of the mesenteric vasculature and not vasodilation is the first event after PVL,[63] this led to the conclusion that upregulation of NO production by eNOS reflects an adaptation to shear stress.[61 63] In fact, the increased NO production by eNOS precedes the development of portal hypertension.[44] It is around day 3 after PVL that this mechanism results in vasodilation.[63] Based on these studies, it is assumed that mild increases in portal pressure, possibly similar to what occurs during early cirrhosis, cause the release of VEGF.[61] The earliest VEGF release in response to PVL starts within hours in the microvasculature of the intestinal mucosa and triggers eNOS dysregulation via the VEGFR-2.[61]

Apart from Ca^{2+}-dependent activation and regulation by phosphorylation, eNOS activity is critically governed by availability of cofactors (table 1). Interestingly, endotoxaemia due to increased bacterial translocation stimulates GTP cyclohydrolase I in mesenteric vessels, leading to increased production of the essential cofactor of eNOS, tetrahydrobiopterin (BH(4)).[64] In studies using antibiotics for intestinal decontamination, it has been demonstrated that increased availability of BH(4) as a result of bacterial translocation contributes to increased eNOS activity in these animals.[49] Interestingly, intestinal bacterial decontamination has been shown to increase vascular resistance and to decrease portal pressure in patients with cirrhosis, highlighting the role of bacterial translocation in portal hypertension.[65] Another mediator with eNOS-activating properties is heat shock protein 90 (Hsp90). Upregulation of Hsp90 and increased interaction of eNOS with Hsp90 contributes to the elevation in NOS activity in mesenteric vessels of PVL rats.[66 67]

It is noteworthy that the increased eNOS activity in hypocontractile vessels persists despite elevated plasma concentrations of asymmetric dimethylarginine (ADMA), an endogenously produced inhibitor of eNOS. Such increased serum levels of ADMA were observed in BDL rats and in patients with alcohol-induced liver cirrhosis,[68 69] but not in PVL rats.[68] Since ADMA contributes to endothelial dysfunction within the intrahepatic microvasculature, but has apparently no effects in extrahepatic vessels, this suggests a different regulation of eNOS by ADMA in different vascular beds. The ADMA-degrading enzyme, dimethylarginine dimethylaminohydrolase (DDAH), represents a possible candidate to account for these discrepancies.[68]

A further source of excessive mesenteric NO production is nNOS, which is expressed in perivascular NANC neurons. Using isolated perfused mesenteric vascular beds, it was demonstrated that nNOS-mediated NO release is increased in response to electric perivascular field stimulation in portal hypertensive rats. This is in accordance with nNOS upregulation in periarterial nerves.[70 71]

NO might induce vasodilation by cGMP-dependent and -independent mechanisms. While cGMP-dependent mechanisms are well characterised for normal conditions and cirrhosis, those of cGMP-independent vasorelaxation by NO have been described only recently.[72–74] To date, no study has focused exclusively on cGMP-independent mechanisms of NO-mediated vasodilation in cirrhosis. However, a previous study provided hints at a putative role,[75] since inhibition of NOS or GC elicited different effects in mesenteric arteries from PVL rats.

Circulating vasodilators

In addition to intracellular eNOS regulation (table 1), the role of circulating hormones for vascular NO overproduction has attracted considerable attention in recent years (table 2). Increased plasma levels of circulating vasodilators were demonstrated in particular in human cirrhosis. Their effect may be NO dependent or NO independent (table 2). A rising body of evidence has revealed the contribution of the endocannabinoid system to the development of extrahepatic vasodilation in cirrhosis. The endocannabinoid system was identified as a local vasomotor system with pronounced effects within the mesenteric vasculature.[76 77] The mechanisms of endocannabinoid-mediated vasorelaxation are various (table 2).[78] Activation of endothelial cannabinoid 1 (CB1) receptors and of vanilloid receptor 1 (VR1) by endocannabinoids (anandamide, 2-arachidonoyl glycerol) causes pronounced vasorelaxation in BDL rats.[79] Increased endocannabinoid production, for example by monocytes, and upregulation of endothelial CB1 receptors contribute to the increased mesenteric NO production by eNOS in mesenteric vessels from portal hypertensive rats.[76 77 79–81] In a similar manner, increased responsiveness to the vasodilator peptide adrenomedullin and its elevated plasma levels are well known to contribute to increased eNOS activity, and thus also to vasodilation, in cirrhotic rats[82–88] and patients.[89] Finally, some studies suggested an increased secretion of ANP which further contributes to increased cGMP levels in cirrhotic patients.[90 91] Another study performed in cirrhotic patients concluded that ANP is not related to haemodynamic changes in cirrhosis. Hence, the exact role of ANP in hyperdynamic circulation and portal hypertension remains to be established.

Non-NO/cGMP-mediated vasodilation

Apart from NO, some evidence for the contribution of vasodilators, in particular of the prostanoid PGI_2, acting via cAMP to vasodilation in cirrhosis

Table 2 Vasodilators mediating splanchnic vasodilation

	Production, occurrence	Mechanisms of vasodilation	(Dys)regulation in cirrhosis
Endocannabinoids (anandamide, 2-AG)	▷ Increased production by monocytes ▷ Circulating hormones	▷ Endothelial release of NO, prostanoids, and/or endothelial-derived hyperpolarising factor ▷ Stimulation of vanilloid receptors on sensory nerves, subsequent release of calcitonin gene-related peptide ▷ Inhibition of VSM cell Ca^{2+} channels ▷ Activation of endothelial and VSM cell receptors ▷ Activation of various VSM cell K^+ channels	▷ Increased production by monocytes ▷ Increased plasma levels of anandamide ▷ Enhanced action by CB1 receptor and VR1 upregulation
Adrenomedullin	▷ Production by endothelial and VSM cells, upon cytokine stimulation ▷ Production in the atrium of the heart ▷ Paracrine and circulating hormone	▷ NO-mediated mechanisms: Ca^{2+}/CaM- and Akt-mediated eNOS activation ▷ VSM cell K^+ channel-mediated mechanisms ▷ Induction of cAMP formation, decrease in $[Ca^{2+}]$ in VSM cells	▷ Increased production in the vessel wall ▷ Increased plasma levels of adenomedullin ▷ Enhanced responsiveness to adrenomedullin
CGRP	▷ Release by neurons ▷ Released by vanilloid receptor stimulation, e.g. after CB1 receptor stimulation	▷ Endothelial release of NO after activation of endothelial CGRP receptors ▷ Induction of cAMP formation after activation of VSM cell CGRP receptors ▷ Activation of endothelial and VSM cell CGRP receptors	▷ Increased production due to anandamide/CB1 receptor-mediated VR1 overactivation
ANP	▷ Cardiac production in response to ventricular overload ▷ Circulating hormone	▷ Induction of cGMP production ▷ Binds to GC-coupled receptors on VSM cells	▷ Increased plasma levels of ANP
Glucagon	▷ Production in pancreas ▷ Circulating hormone	▷ Receptor/G-protein mediated AC activation, induction of cAMP formation in VSM cells	▷ Increased plasma levels of glucagon
Carbon monoxide (CO)	▷ Production in the vessel wall by HO-1 ▷ Paracrine/autocrine factor	▷ Activation of soluble GC, induction of cGMP production	▷ Increased production in the vessel wall due to upregulation of HO-1 expression
PGI_2	▷ Production by COXs ▷ Released from endothelial cells	▷ Activation of AC, induction of cAMP formation in VSM cells	▷ Upregulation of COX expression ▷ Increased plasma levels of PGI_2

AC, adenylyl cyclase; 2-AG, 2-arachidonoyl glycerol; ANP, atrial natriuretic protein; CaM, calmodulin; CB1, endothelial cannabinoid 1; CGRP, calcitonin gene-related peptide; COX, cyclo-oxygenase; eNOS, endothelial nitric oxide sythase; GC, guanylyl cyclase; HO-1, haem oxygenase-1; NO, nitric oxide; PGI_2, prostacyclin; VR1, vanilloid receptor 1; VSM, vasular smooth muscle.

was also provided (table 2). PGI_2 acts via prostaglandin receptors at VSM cells. The synthesis of PGI_2 involves cyclo-oxygenases (COXs), of which two isoforms have been described. Both COX isoforms, the constitutively expressed COX-1 and the inducible COX-2, are upregulated in hypocontractile vessels of PVL rats.[92 93] This possibly explains the increased production of prostanoids, which contributes to endothelium-dependent vasodilation in BDL rats.[94] However, such changed activities of PGI_2-synthesising enzymes were not found in aortas from PVL rats,[95] despite enhanced PGI_2 synthesis,[94] at least in response to adrenaline.[95] Nevertheless, the arterial cAMP content is elevated in PVL rats,[34] despite the fact that the mechanisms causing this elevation apparently differ from those in other animal models. Increased cAMP formation in hypocontractile vessels might also occur independently of endothelium from elevated levels of circulating glucagon. Glucagon induces cAMP production in response to activation of $G\alpha_s$/AC-coupled GPCRs at the VSM cell. In contrast, it seems surprising that basal and G-protein-mediated AC activity were reported to be reduced in dilated preportal vessels from PVL rabbits, due to upregulation and increased activity of AC-inhibiting $G\alpha_i$-proteins.[96] Although it is not known whether the same holds true for other species or other models of portal hypertension, the discrepancy between these findings and those of other studies apparently demonstrates that other mechanisms (eg, increased levels of AC-stimulating mediators) are obviously capable of overcoming such a possible G-protein-mediated decrease in cAMP formation in hypocontractile vessels. Thus, the exact role of cAMP-dependent and $G\alpha_{i/s}$-governed pathways for vasodilation and portal hypertension in cirrhosis remains to be elucidated.

In recent years, increased expression and activity of haem oxygenases (HOs) was described to contribute to impaired vasocontraction and vasodilation in BDL,[97] CCl_4[98 99] and PVL rats (table 2).[100] In this scenario, carbon monoxide (CO) produced by HO during haem metabolism to biliverdin IX is believed to induce vasorelaxation by activation of NO-dependent GC. However, this is somewhat unexpected, since it is well known that CO acts as only a very weak activator of this GC (3- to 6-fold activation compared with the 100- to 200-fold activation induced by NO at the purified enzyme).[101 102] Furthermore, it remains to be explained how these mechanisms contribute to vasodilation despite the fact that biliverdin IX is an endogenous inhibitor of guanylate cyclase,[103] and regardless of the possibility that HO might also metabolise the haem moieties of NO-sensitive GC

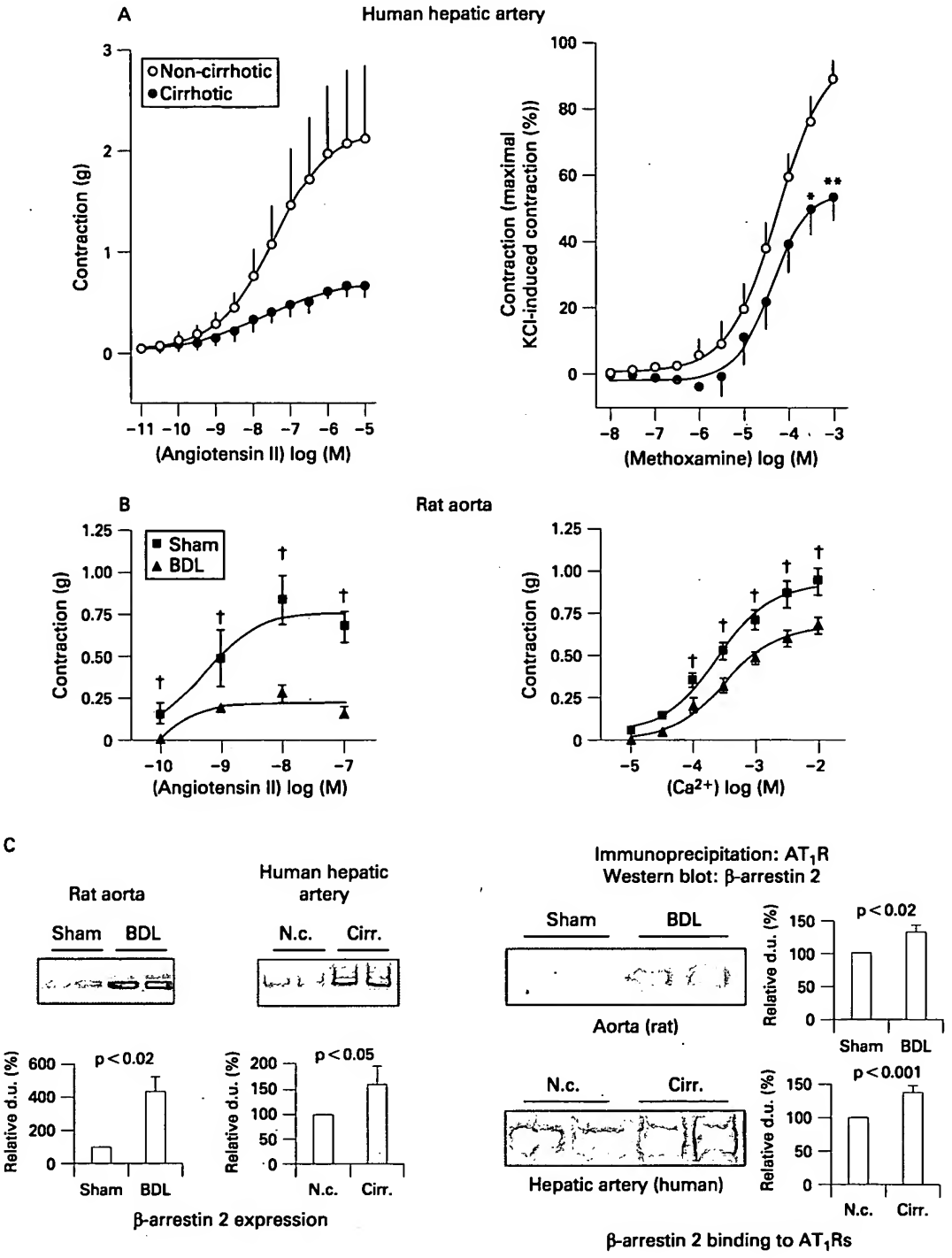

Figure 5 Endothelium-independent vasoconstrictor hyporesponsiveness and increased binding of β-arrestin 2 to the angiotensin II type 1 receptor (AT_1R) in hypocontractile vessels. (A) Extrahepatic segments of hepatic arteries from patients with cirrhosis are hypocontractile to angiotensin II and the α_1-adrenoceptor agonist, methoxamine, despite endothelium denudation. (B) Similarly, isolated aortic rings from cirrhotic bile duct ligated (BDL) rats show nitric oxide-independent hyporesponsiveness to angiotensin II and diminishment of Ca^{2+} sensitivity. (C) The hyporesponsiveness to angiotensin II is associated with overexpression of the receptor-desensitising protein, β-arrestin 2, and increased binding of β-arrestin 2 to AT_1R in vessels from patients with cirrhosis of the liver and rats with experimentally induced cirrhosis of the liver. (Adapted from Hennenberg *et al*[121], Heller *et al*[127], Schepke *et al*[129]). Cirr., cirrhotic; d.u., densitometric units; N.c., non cirrhotic.

and eNOS, which are required for their activation. This strongly suggests that further mechanisms and events are involved in CO-mediated vasodilation in cirrhosis which remain to be fully understood.

Vascular tone is also determined by ATP-sensitive K^+ channels (K_{ATP} channels). Opening of K_{ATP} channels in VSM cells causes hyperpolarisation and subsequent vasorelaxation (fig 3). Experiments using K_{ATP} channel modulators suggested that in dilated vessels in cirrhosis, K_{ATP} channels might already be maximally opened in PVL and BDL rats.[75 104–107] Furthermore, this is apparently related to endothelium-derived factors. Interestingly, in vessels from normal rats, the non-selective endothelin receptor antagonist bosentan elicited similar effects to endothelium removal, whereas exogenous endothelin mimicked the situation observed in vessels from BDL rats.[105] Thus, changes in paracrine signalling by endothelin were concluded to be responsible for the altered control of extrahepatic vascular tone by K_{ATP} channels in BDL rats.

Interestingly, ATP-sensitive K^+ channels are also a target of hydrogen sulfide (H_2S), recently identified as a potent gaseous mediator of vasodilation.[108] The H_2S-induced vasodilation is explained

by stimulation of K_{ATP} channels, but does not stimulate GC.[108] While a role for H_2S in K_{ATP} channel activation and vasodilation in cirrhosis was proposed theoretically,[109] this has not been investigated to date.

The molecular basis for endothelium-derived hyperpolarising factor (EDHF) is still unknown. Several candidates have been discussed.[110] EDHF might compensate for blocked NO- and COX-mediated vasodilation during acetylcholine-induced relaxation of mesenteric arteries from BDL rats with NOS/COX inhibition.[111] However, whether this plays a role in vivo remains unclear.

Findings on a possible role for the vasodilator adenosine in vasodilation are conflicting. While the adenosine response may be enhanced in the splanchnic vascular bed and gastric microcirculation of BDL rats,[112 113] other authors found a diminished responsiveness to adenosine or antagonists in mesenteric arteries from PVL rabbits and in vivo in PVL or BDL rats.[114–116] Thus, despite several studies, the role of adenosine in vasodilation remains elusive.

Impaired contractile signalling in vascular smooth muscle cells

Increased NO production and increased actions by vasodilators can only partially explain vasodilation and vascular hypocontractility in cirrhosis. In all animal models of cirrhosis, a component of vasoconstrictor hyporesponsiveness was found in isolated vessels, which is resistant to endothelium denudation and pharmacological NOS inhibition.[21 85 117–126] Endothelium-independent hyporeactivity to vasoconstrictors was also described for isolated human hepatic arteries from cirrhotic patients (fig 5).[127–129] Additionally, in vivo studies with NOS inhibitors also suggested the existence of NO-independent vasodilation in cirrhotic and portal hypertensive rats[130 131] and patients.[132] Further evidence for NO-independent vasodilation in portal hypertension comes from eNOS knockout mice, which develop hyperdynamic circulation in animal models of portal hypertension.[133–135] eNOS knockout mice showed increased portal tributary flow after PVL.[135]

In many of these studies, be it in animal models or human material, the vascular responsiveness to α_1-adrenoceptor agonists (phenylephrine, methoxamine, noradrenaline) was assessed. In isolated perfused mesenteric vascular beds of PVL,[117 119] BDL[117] and CCl_4 rats,[125] in isolated aortic rings of these animals,[21 120 122 123 125 126] or in isolated hepatic arteries from cirrhotic patients,[127] the diminished contractile response to α_1-adrenergic agonists persisted after removal of the endothelium or pharmacological inhibition of endogenous NO production (fig 5). Similar observations were made for other vasoconstrictors, in particular for angiotensin II,[120 121 129] endothelin 1[118] and vasopressin (fig 5).[123 128] Thus, vasodilator-independent reduction of the vasopressor response is a common feature of vasodilation in human and experimental cirrhosis.

Based on evidence obtained from experiments with L-type Ca^{2+} channel activators,[136] or when using high molar potassium which causes depolarisation and subsequent opening of voltage-gated Ca^{2+} channels,[21] it can be excluded that the vasodilator-independent hyporeactivity to vasoconstrictors is related to any changes in signalling induced by the capacitative Ca^{2+} influx after opening of these channels, or occurs due to a loss of contractile proteins.

Although downregulation of vasopressor receptors or their associated G-proteins would represent a simple explanation for such an impaired vascular responsiveness to vasoconstrictors, evidence suggesting reduced receptor expression has not been provided so far. In hepatic arteries from cirrhotic patients, mRNA levels of α_1-adrenoceptors, the AT_1R, the V_1 receptor and endothelin receptors remained unchanged or were even increased.[137] Accordingly, radioligand binding to α_1-adrenoceptors is unaltered in aortas from BDL rats[138] and mesenteric arteries from PVL rats.[139] In another study, the expression of the AT_1R and associated Gα-proteins ($G\alpha_{q/11}$, $G\alpha_{12}$, $G\alpha_{13}$) was unchanged in aortas from BDL rats and in hepatic arteries from cirrhotic patients.[121]

Recent evidence suggests that impairment of contractile signalling occurs already early after receptor stimulation, most probably at the level of Gα effectors. In BDL rats, impaired reactivity to

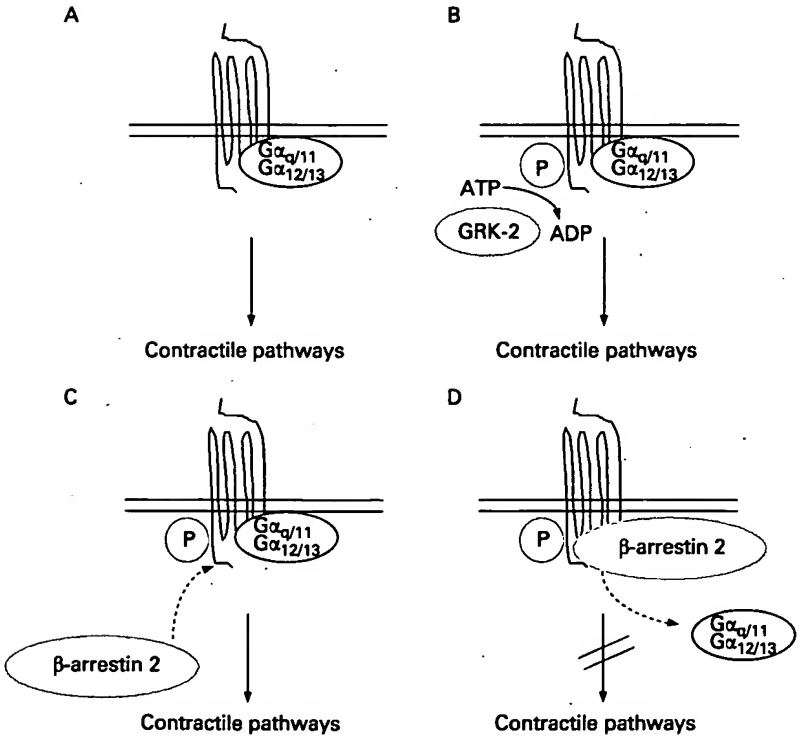

Figure 6 Desensitisation of G-protein-coupled receptors (GPCRs) by GPCR kinase 2 (GRK-2) and β-arrestin 2. Ligand binding to GPCRs, including α_1-adrenoceptors and the angiotensin II type 1 receptor, activate GRK-2. As a result, GPCRs may be phosphorylated by GRK-2. This enhances the binding of β-arrestin 2 to the receptors. β-Arrestin 2 replaces receptor-associated heterotrimeric G-proteins, resulting in desensitisation of the receptor.

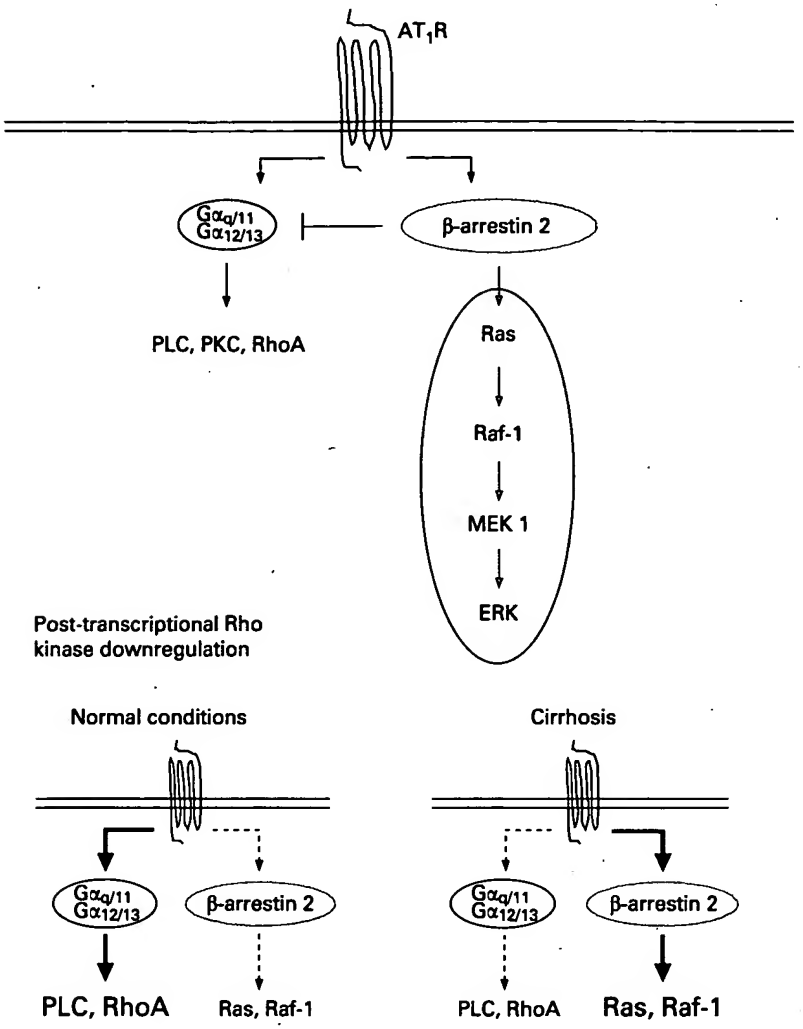

Figure 7 Assumed β-arrestin 2-mediated switching of the angiotensin II type 1 receptor (AT_1R) in cirrhosis. Apart from G-protein-dependent pathways (eg, contraction pathways), the AT_1R couples to alternative pathways, which are activated G-protein-independently, but via β-arrestin 2. The figure summarises the current model of receptor switching which may underlie vascular hypocontractility in cirrhosis. Under normal conditions, extrahepatic vascular AT_1Rs predominantly couple to G-protein-dependent pathways, resulting in Rho kinase-, protein kinase C (PKC)- and Ca^{2+}-mediated contraction and $G\alpha_{q/11}$/PKC-mediated activation of the mitogen-activated protein (MAP) kinase, extracellular signal-regulated kinase 1/2 (ERK1/2). In cirrhosis, however, the AT_1R switches to β-arrestin 2-mediated signalling pathways, which are now preferentially activated instead of the G-protein scaffolded cascades, due to enhanced interaction between AT_1Rs and β-arrestin 2. This causes blunting of contraction and activation of non-nuclear ERK. The changed mode of ERK activation possibly accounts for the post-transcriptional downregulation of Rho kinase in these vessels as post-transcriptional Rho kinase downregulation by MAP kinase kinase (MEK)/ERK has been reported from cultured fibroblasts. Since other vasoconstrictor receptors (eg, α_1-adrenoceptors) display similar coupling patterns, these processes might be relevant not only for the AT_1R, but also for other G-protein-coupled vasoconstrictor receptors. PLC, phospholipase C.

α_1-adrenoceptor stimulation includes reduced activation of PLC and consecutively diminished formation of inositol phosphates,[123] as well as reduced activation of RhoA with subsequently defective Rho kinase activation.[21 121] Impairment of PLC activation not only causes diminished IP_3 formation and impaired Ca^{2+}-dependent contraction, but also leads to decreased DAG synthesis. Thus, decreased PLC activation possibly also explains the impaired activation of DAG-dependent PKC isoforms, which has been described from aortas from BDL rats.[140 141] Impaired signalling by RhoA and Rho kinase in turn results in decreased phosphorylation of Ca^{2+}-sensitising proteins, increased MLC phosphatase activity and decreased Ca^{2+} sensitivity.[121] Importantly, the reduction in PLC and RhoA activation was resistant to endothelium denudation or pharmacological NOS inhibition.[21 123] Together, this suggests the existence of defects in receptor/G-protein-mediated activation of contraction cascades within VSM cells of hypocontractile vessels from cirrhotic species.

The impaired response to angiotensin II in aortas from BDL rats and in hepatic arteries from cirrhotic patients is associated with upregulation of the expression of receptor-desensitising proteins, namely G-protein-coupled receptor kinase 2 (GRK-2) and β-arrestin 2, and with increased binding of β-arrestin 2 to the AT_1R in these vessels (fig 5).[121] As expression of the AT_1R and its associated α-subunits of heterotrimeric G-proteins ($G\alpha_{q/11}$, $G\alpha_{12}$, $G\alpha_{13}$) is unaltered, this suggests that the AT_1R in these vessels is most probably desensitised by the concerted action of GRK-2 and β-arrestin 2. In such a process of receptor desensitisation (fig 6), GPCRs are first phosphorylated through GRK-2 after receptor stimulation. This GRK-2-mediated receptor phosphorylation enables β-arrestin 2 to bind to the receptors. As a result, heterotrimeric G-proteins dissociate from their associated receptors, resulting in uncoupling and desensitisation of the receptor.[142–148] Under normal conditions, these mechanisms counteract exaggerated receptor stimulation.

The GRK-2/β-arrestin 2 system induces desensitisation of a variety of different receptors.[142–148] It is tempting to assume that β-arrestin 2-mediated receptor desensitisation not only occurs at the AT_1R—as shown by us—but also at other vasopressor receptors, including α_1-adrenoceptors. AT_1Rs and α_1-adrenoceptors are both coupled to the same contractile pathways, so that they use identical mechanisms for vasocontraction. As the β-arrestin 2-mediated receptor desensitisation is initiated in response to exaggerated receptor stimulation, it seems possible that elevated plasma levels of angiotensin II and catecholamines, which are well established in cirrhosis,[6–9 120] are responsible for the onset of these processes in hypocontractile vessels.

In another interesting study, it was found that RhoA is downregulated in mesenteric arteries from PVL rats at the mRNA, but not at the protein level.[149] Parallel to this, a decrease in membrane-associated RhoA was found in these vessels, reflecting diminished amounts of active RhoA. This was normalised by acute PKA inhibition. As PKA inhibition was without effect on RhoA expression, the authors concluded that cAMP-dependent events are responsible for the restoration of RhoA membrane association in mesenteric vessels of PVL rats.

In addition to the impaired agonist-induced activation of contraction cascades, defective constitutive contractile signalling was also observed

under resting conditions. Thus, isolated aortic rings from BDL rats with pharmacologically blocked NO production show a decreased Ca^{2+} sensitivity even in the absence of any vasoconstrictors (fig 5).[121] This is paralleled by a reduction in constitutive phosphorylation of Rho kinase substrates, including the mediators of Ca^{2+} sensitisation, MYPT1 and CPI-17. This, in turn, is associated with a decreased basal Rho kinase activity due[21 121] to post-transcriptional downregulation of the enzyme.[21] Post-transcriptional Rho kinase downregulation was also noted in mesenteric vessels of BDL rats.[150] Moreover, Rho kinase downregulation and decreased levels of constitutively phosphorylated Ca^{2+}-sensitising proteins and other Rho kinase substrates were also observed in hepatic arteries from cirrhotic patients.[121] Finally, MLC phosphatase activity is indeed elevated in aortic homogenates from BDL rats.[121]

Further evidence for reduced Rho kinase activity leading to reduced vascular tone and vasodilation comes from haemodynamic studies performed in BDL rats. Application of the Rho kinase inhibitor Y-27632 demonstrated a substantial contribution of Rho kinase to the regulation of splanchnic and systemic vascular resistance in normal rats, which was considerably blunted in BDL rats by Y-27632.[21 151]

In conclusion, Rho kinase downregulation represents a further mechanism which contributes to defective contractile signalling and vasodilation in cirrhosis parallel to the impaired vasoconstrictor-induced activation of Rho kinase.

Results from cell culture studies suggest that the elevated angiotensin II plasma levels together with β-arrestin 2 overexpression in cirrhosis not only cause receptor desensitisation, but are also responsible for Rho kinase downregulation. In cultured fibroblasts, overactivation of Ras/Raf/MEK (mitogen-activated protein kinase kinase) which is also partially coupled via β-arrestin 2 to the AT_1R,[152–154] caused post-transcriptional downregulation of Rho kinase.[155] Thus, elevated plasma levels of angiotensin II in combination with overexpression of β-arrestin 2 might impair contractile signalling in vascular smooth muscle via different mechanisms (fig 7). First, binding of β-arrestin 2 to receptors causes desensitisation of G-protein-dependent signalling, including vasoconstrictor-induced Rho kinase activation. Secondly, enhanced interaction between receptors and β-arrestin 2 might result in overactivation of β-arrestin 2-dependent signalling, and subsequently exaggerated β-arrestin 2-mediated extracellular signal-regulated kinase (ERK) activation may be responsible for post-transcriptional downregulation of Rho kinase expression. Thus, this suggests a switching of AT_1R coupling in vascular hypocontractility, namely from G-protein-dependent pathways as the predominantly activated signalling under normal conditions, to β-arrestin 2-dependent pathways which might be preferentially activated during cirrhosis (fig 7).

Hypophosphorylation of CPI-17 at Thr38 and subsequently decreased Ca^{2+} sensitivity in hypocontractile vessels may also come from decreased activity and impaired activation of several isoforms of PKC. The conventional isoforms of PKC are DAG dependent. DAG is synthesised in parallel to IP_3 by PLC, after receptor stimulation by vasoconstrictors. Decreased activation of PKCα has been reported from aortas from BDL rats,[139 140] and might be at least partially related to decreased PLC activity due to receptor desensitisation. However, since direct receptor-independent activation of PKCα by phorbol esters was also impaired in these studies,[140] further mechanisms obviously contribute to the impairment of PKC activation.

Another interesting example for dysregulation of contractile signalling in portal hypertension was provided recently. Under normal conditions, sympathetic α_1-adrenergic vasocontraction may be enhanced by neuropeptide-Y (NPY), which is released together with catecholamines and acts via the G-protein-coupled Y1 receptor on VSM cells.[156] The pronounced effect of exogenous NPY on α_1-adrenergic mesenteric contraction in PVL rats suggested in vivo a lack of amplification of contractile signalling by the absence of NPY in these vessels.[157] Yet, the relevance of elevated NPY levels at least in advanced human cirrhosis remains to be clarified in this context.[158] Nevertheless, these studies underline the importance of contractile signalling in VSM cells, which may be endothelium-independently dysregulated at different levels in portal hypertension. It will be exciting to determine to what extent these mechanisms of vasodilation are also found in animal models of cirrhosis with portal hypertension.

Table 3 Factors contributing to extrahepatic vasodilation in cirrhosis

	Mediators, effectors	Mechanisms
Defective activation of contractile signalling	β-Arrestin-2, GRK-2 Rho kinase	▷ Desensitisation of vasoconstrictor receptors ▷ Rho kinase downregulation
Increased endothelial NO production	eNOS NO cGMP/PKG	eNOS upregulation Dysregulation of eNOS
Circulating vasodilators	▷ Circulating mediators: adrenomedullin, glucagon, endocannabinoids ▷ Cyclo-oxygenases, haem oxygenase, eNOS ▷ cAMP, cGMP	▷ Vasodilation via increased plasma concentrations ▷ Upregulation of effectors
Neuronal vasodilation	▷ Perivascular NANC neurons ▷ nNOS	▷ Increased NO/cGMP-mediated vasorelaxation

eNOS, nitric oxide synthase; GRK-2, G-protein-coupled receptor kinase 2; NANC, non-adrenergic non-cholinergic; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; PKG, cGMP-dependent kinase.

CONCLUDING REMARKS

Vascular hypocontractility and vasodilation in cirrhosis is a multifactorial phenomenon (table 3). Several mechanisms have been identified so far, which contribute to vasodilation and impaired vasocontraction. These include overproduction of vasodilators and impaired responsiveness to vasoconstrictors. Although NO overproduction is widely accepted as a main culprit of vasodilation in cirrhosis, several studies including those in eNOS

knockout mice have shown that other factors besides NO are involved in the pathogenesis of arterial vasodilation. Another mechanism seems to be defective contractile signalling in smooth muscle cells in response to vasoconstrictor stimulation. However, little is known about the governing factors (besides liver disease itself) responsible for the development of these defects, and about possible interactions between participating pathways and mechanisms. Thus, further efforts are necessary to improve our understanding of the primary events causing vasodilation, and to identify superior, common factors responsible for the different defects in vasomotoric signalling.

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Editor's quiz: GI snapshot

ANSWER

From the question on page 1260

Pneumatosis ventriculi was detected as a linear accumulation of gas within the gastric wall. There were no signs of free intraperitoneal air or fluid. The histologically confirmed carcinoma of the gastric antrum could not be delineated in the computed tomography sections. No enlargement of the regional lymph nodes was observed.

Compared to the pneumatosis of the intestinum the pneumatosis ventriculi is a very rare clinical condition, which can be identified by computed tomography. For the differentiation between intramural air and lipomatosis the lung window view can be helpful. Aetiological factors for pneumatosis ventriculi include bacterial infection, increased intragastric pressure, infiltration by intra-abdominal malignancies,[1] mucosal injury[2] or bowel ischaemia.[3] In the case presented an iatrogenic origin caused by the biopsy during the diagnostic oesophagogastroduodenoscopy was the assumed trigger. After uncomplicated subtotal gastric resection with lymphadenectomy and the regular postoperative period the patient was discharged in good clinical condition.

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Vascular Deterioration in Cirrhosis

The Big Picture

Jaime Bosch, MD, PhD

Abstract: Cirrhosis is characterized by marked abnormalities in the hepatic circulation. Functionally, there is an increased vascular tone and impaired flow-mediated vasorelaxation, whereas anatomically there is sinusoidal remodeling and capillarization, angiogenesis, venous thrombosis, and vascular distortion, all contributing to increase hepatic vascular resistance and portal hypertension. However, vascular changes are not limited to the liver, but are also present in the splanchnic organs, heart, lungs, kidney, brain, and skin. Advances in the knowledge of the mechanisms of these abnormalities have disclosed new targets for therapy and ultimately improved survival.

Key Words: portal hypertension, variceal bleeding

(*J Clin Gastroenterol* 2007;41:S247–S253)

CIRRHOSIS: A VASCULAR DISEASE OF THE LIVER (BUT NOT ONLY)

Vascular abnormalities are ubiquitous in cirrhosis. It has long been known that cirrhosis may be considered as a vascular disease of the liver, owing to the marked anatomic changes that occur at the intrahepatic circulation. These determine an increased mechanical resistance to portal blood flow and an increased hepatic vascular tone, both leading to portal hypertension and its complications.[1–3] In addition, distortion of the hepatic circulation, with sinusoidal remodeling and formation of intrahepatic shunts has important functional consequences interfering with metabolic exchange between the hepatocytes and sinusoidal blood flow and decreasing O_2 transport to the hepatocyte; as a result, liver function further deteriorates and liver injury is aggravated.

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A Splanchnic Vascular Disease (Fig. 1)

Cirrhosis further causes marked abnormalities in the splanchnic vascular bed. These include functional changes, such as splanchnic vasodilation and decreased responsiveness to vasoconstrictors, and structural changes, such the formation of new blood vessels (angiogenesis), which includes an arteriolar-capillary network to support the increased splanchnic blood flow, and the formation of portal-systemic collaterals.[4] The former contribute to further increase portal pressure, the later are responsible for the formation of *gastroesophageal varices, portal hypertensive gastropathy* and colopathy, variceal bleeding episodes, and portal-systemic shunting-related complications. Increased portal pressure also plays a central role leading to *splenomegaly*, thrombocytopenia, leucopenia, and anemia ("hypersplenism").

A Systemic Vascular Disease (Fig. 1)

The systemic circulation is characteristically altered in cirrhosis. This occurs after the development of portal hypertension, which is associated with a distinctive systemic circulatory abnormality known as *the hyperkinetic syndrome*, which is characterized by hypervolemia, increased cardiac index, hypotension and decreased systemic vascular resistance.[5] The hyperkinetic syndrome contributes to aggravate portal hypertension (by increasing the portal venous inflow) and plays a central role in the pathogenesis of ascites and renal dysfunction in chronic liver disease.[6] The hyperkinetic syndrome leads to a situation of hypotension and "effective" hypovolemia, volume receptors being stimulated despite the increased circulating blood volume. This leads to a marked activation of neurohumoral vasoactive factors in an attempt to maintain the arterial blood pressure within normal values.

A Vascular Disease With Multiorgan Involvement (Fig. 1)

The Kidney

Renal abnormalities in cirrhosis are mostly functional, and characterized by marked renal vasoconstriction, responsible for the development of *hepatorenal syndrome*. Renal vasoconstriction develops as a consequence of the splanchnic vasodilation and systemic hyperkinetic syndrome, in response to effective hypovolemia and ensuing neurohumoral activation.[6] These concepts had provided the rationale for treating the

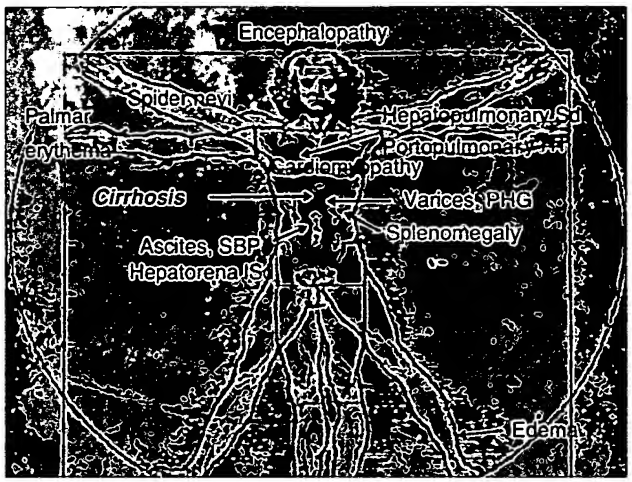

FIGURE 1. Vascular deterioration in cirrhosis: the big picture.

hepatorenal syndrome with albumin infusion and vasoconstrictors (terlipressin, norepinephrine, or midodrine).[7]

The Lung

Vasodilatation in the lung leads to ventilation perfusion mismatch and even to A-V shunts in the pulmonary circulation; these determine the *hepatopulmonary syndrome*, characterized by marked hypoxemia.[8,9] In some cases, this may evolve into the opposite situation, with markedly increased pulmonary vascular resistance in the *portopulmonary hypertension*.[10] The later is thought to develop through endothelial dysfunction and vascular remodeling of the pulmonary circulation.[11]

The Brain

Changes in cerebral blood flow and vascular reactivity associated with portal hypertension are thought to contribute to facilitate some of the brain abnormalities of hepatic encephalopathy.

The Heart

So-called cirrhotic cardiomyopathy is characterized by some electrocardiogram abnormalities such as QT prolongation, although this is in part due to the tachycardia commonly associated to the hyperkinetic syndrome. In terminal stages of cirrhosis, especially in patients with sepsis and/or hepatorenal syndrome, the hyperkinetic syndrome disappears and the patients rather exhibit a decreased cardiac index. This is of clinical relevance because it probably contributes to further aggravate renal failure.[12] To what extent this is caused by the cirrhotic cardiomyopathy or by the release of cardiodepressing cytokines prompted by sepsis remains uncertain.

The Skin

Advanced cirrhosis is characterized by warm skin, bounding pulses, and palmar erythema, which all reflect the participation of the peripheral circulation in the hyperkinetic syndrome. In addition, dermal angiogenesis gives rise to the spider angioma characteristic of advanced liver disease. Hepatopulmonary syndrome may cause finger clubbing.

MAIN PATHOPHYSIOLOGIC EVENTS

Inflammation, Oxidative Stress, Insulin Resistance

These are thought to be the main mechanisms of liver injury in most diseases that may evolve into cirrhosis. The importance of these events goes beyond causing liver injury; indeed, all are involved in many of the functional abnormalities encountered in cirrhosis, that go from abnormal gene regulation to decreased protein synthesis. As discussed below, this may be of critical relevance in some of the abnormalities of the intrahepatic circulation contributing to portal hypertension in cirrhosis (Fig. 2).

Sinusoidal Endothelial Dysfunction

Cirrhosis is associated with increased hepatic vascular tone and impaired endothelial dependent vasorelaxation in response to flow or to pharmacologic stimuli. These abnormalities define what in vascular pathobiology is known as "endothelial dysfunction," whereby the endothelium loses its vasodilatory, antithrombotic, and antiproliferative properties to acquire a vasoconstrictor, prothrombotic, and proliferative phenotype.[13,14] As such, sinusoidal endothelial dysfunction is of key relevance both in the development of portal hypertension (in which the increased hepatic vascular tone is thought to account for about one third of the increase in hepatic vascular resistance) and in disease progression.[15] Because of that, sinusoidal endothelial dysfunction represents a rational target for therapy.

The major effectors responsible for the consequences of endothelial dysfunction are an imbalance between decreased release of endothelial vasodilators

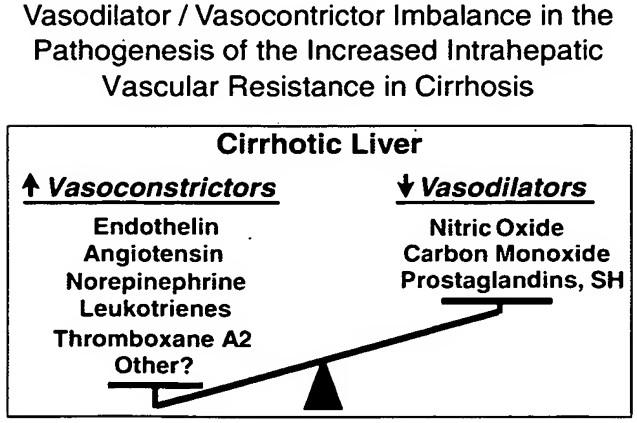

FIGURE 2. Factors increasing hepatic vascular tone in cirrhosis.

and increased production of endothelial vasoconstrictors (Fig. 2).

Nitric oxide (NO) is the main endothelial vasodilator. In cirrhosis, sinusoidal NO production is insufficient to prevent an increased hepatic vascular tone.[15] This is not due to reduced expression of endothelial NO synthase (eNOS), but to a decreased eNOS activity due to a myriad of postranslational regulatory defects, including increased caveolin interaction,[16,17] decreased phosphorilation,[18,19] eNOS uncoupling by diminished BH4 availability,[20] scavenging of NO by superoxide anions to form peroxynitrite,[21] and increased levels of asymmetric d-methyl-arginine and other eNOS inactivators.[22] Each of these defects may be corrected therapeutically, which is currently a hot topic for research.

Other endothelial dilators that may be deficient in cirrhosis and thus contribute to sinusoidal endothelial dysfunction are CO and H_2S, but available data on their relevance are scarce.[23]

Increased release of vasoconstrictive prostanoids has been shown to play a relevant role favoring sinusoidal endothelial dysfunction and increasing hepatic vascular tone. The most important are the COX-1–dependent constrictors thromboxane A2 and the cysteinyl leukotrienes.[24,25]

Hepatic Stellate Cell Activation and Sinusoidal Remodeling

The hepatic stellate cells (liver specific pericytes) exhibit an activated phenotype in liver disease and, among other features, acquire contractile properties (by which HSC contribute to regulate sinusoidal blood flow and vascular resistance) and synthesize extracellular matrix and collagen. The first consequence is the capillarization of the sinusoids that loose their fenestrae develop a basement membrane and become more rigid, which impairs the metabolic exchange with the hepatocytes and increases mechanically the sinusoidal vascular resistance. The active contraction of the HSC further increases the hepatic vascular tone and contributes to the development of portal hypertension.[26,27] In parallel with this sinusoidal remodeling, there is a process of angiogenesis, mainly along areas of active inflammation and fibrous septa. Angiogenesis probably favors inflammation, tissue repair (and scarring), and gives rise to intrahepatic shunts (Fig. 3).

The above considerations point to several potential new targets for the treatment of portal hypertension (Table 1).

Angiogenesis: Its Role in Splanchnic Hyperemia and in Collateral Formation

Recent studies have shown that portal hypertension is associated with a marked activation of angiogenesis. This is driven by overexpression of vascular endothelial growth factor (VEGF) and VEGF-receptor 2, platelet-derived growth factor (PDGF), and placental growth factor. The VEGF and PDGF pathways are the better studied at present.[4,28,29]

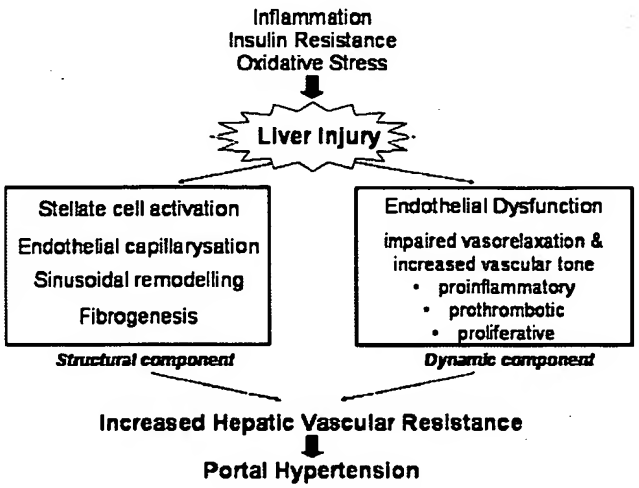

FIGURE 3. From liver injury to portal hypertension: structural and functional changes increasing hepatic resistance in cirrhosis.

VEGF overexpression promotes splanchnic angiogenesis, with formation of new vessels in splanchnic organs and mesentery. Stabilization of these newly formed blood vessels is supported by activation of PDGF, which has a delayed peak with regard to that of VEGF. Besides its involvement in sinusoidal remodeling and fibrogenesis, angiogenesis plays 2 important additional roles in portal hypertension (Fig. 3).

First, in the splanchnic arteriolar territory, the newly formed blood vessels contribute to maintain the increased blood flow to splanchnic organs associated with

TABLE 1. New Targets for the Treatment of Portal Hypertension

Target	Mechanism	Drugs
Cirrhosis	HCV, HBV	Interferons, antivirals
	Alcohol, NASH	Abstinence, weight loss, antioxidants
	Hemochromatosis	Phlebotomy
	Autoimmune	Corticosteroids
Vascular thrombosis	Thrombophilic disorders	Anticoagulation
Increased hepatic vascular tone	Decreased NO release	NO donors (liver specific)
		NOS/aAKT gene transfer
		Statins
		BH4
		Antioxidants
	Increased TX-A2	Thromboxane inhibitors
		COX-1 inhibitors
	Increased endothelin	ET antagonists
Angiogenesis	Increased VEGF, PDGF	Monoclonal antibodies
		VEGF-R2 antagonists
		Mixed VEGF/PDGF blockers

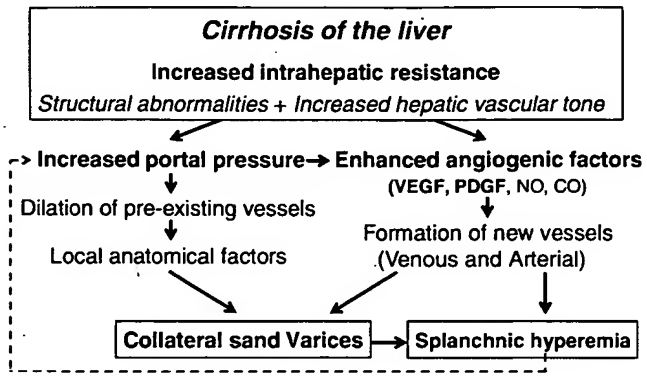

FIGURE 4. Factors leading to the formation of portosystemic collaterals and gastroesophageal varices.

chronic portal hypertension. Studies blocking VEGF by a variety of approaches show that this is associated with decreased portal venous inflow (Fig. 4). The combined blockade of VEGF and PDGF results in a marked fall in portal pressure. Second, in the splanchnic venous territory, VEGF-driven angiogenesis contributes to the formation of portal-systemic collaterals and to the maintenance of portal-systemic shunting. This is a new concept, challenging the traditional hypothesis that formation of collaterals (including varices) was the result of the dilation by the increased portal pressure of preexisting but functionally closed vascular channels at sites of communication between the portal and systemic circulation. It is worth noting that even in conditions not decreasing portal pressure VEGF blockade may prevent collateralization by over 50%.[4,28] Therefore, antagonizing angiogenesis may represent a new therapeutic target for portal hypertension.[30,31]

THE PORTAL HYPERTENSION SYNDROME

Definition and Relevance

Portal hypertension is a common clinical syndrome, which is characterized by a pathologic increase of the portal pressure gradient (the pressure difference between the portal vein and the inferior vena cava) and by the formation of portal-systemic collaterals that shunt part of the portal blood flow to the systemic circulation bypassing the liver.[2] Normal values of the portal pressure gradient ranges from 1 to 5 mm Hg. To become clinically significant (associated with risk of clinical complications), the portal pressure gradient shall increase to 10 mm Hg or above. Values between 5 and 9 mm Hg represent preclinical portal hypertension.[32–35]

The most common cause of portal hypertension worldwide is chronic liver disease (cirrhosis of the liver), followed by schistosomiasis. Other causes of portal hypertension account for less than 10% of cases; among these relatively uncommon causes, the more frequent is portal vein thrombosis. Unless otherwise stated, all following comments pertain to patients with cirrhosis. In them, portal pressure gradient is measured by the hepatic venous pressure gradient (HVPG).[36]

The relevance of the portal hypertension syndrome is due to the frequency and severity of its complications. These include gastroesophageal varices and bleeding, ascites, spontaneous bacterial peritonitis, hepatorenal syndrome, portal hypertensive gastropathy and colopathy, hyperkinetic syndrome, hepatopulmonary syndrome, portopulmonary hypertension, cardiomyopathy, splenomegaly, thrombopenia, leucopenia and anemia, hepatic encephalopathy, increased susceptibility to systemic infections and disturbances in the metabolism of endogenous and exogenous xenobiotics. The complications of portal hypertension represent the first cause of hospital admission, death, and liver transplantation in patients with cirrhosis.

Natural History

Portal hypertension is an almost unavoidable complication of cirrhosis, developing in most patients surviving enough. In perfectly compensated patients at the time of diagnosis, 80% of patients already have an increased portal pressure, and almost 40% have developed varices. Formation of varices occurs at a rate of 10% to 14% per year in patients with a HVPG of at least 10 mm Hg, and of approximately half in those with less pronounced increases in HVPG.[33] When varices are present, the risk of bleeding is relatively high (from 10% to 20% at 1 y) when the patient has "high-risk" factors: HVPG > 12 mm Hg, large varices, presence of red color signs over the varices, and advanced liver failure, whereas the risk of bleeding is half in the remaining ("low-risk" patients).[37] Variceal bleeding carries a high immediate mortality: this is of about 20% at 6 weeks and of 60% at 1 year. Patients surviving variceal bleeding have a high risk of rebleeding, of about 62% at 2 years.[38–40]

The above data illustrate that prognosis of cirrhosis worsens with portal pressure elevation. Indeed, HVPG is a very strong prognostic indicator in these patients. This emphasizes the concept that decreasing portal pressure will result in the prevention/correction of the complications of portal hypertension and in improved survival. This actually has been demonstrated by several studies, underscoring the importance of developing effective treatments for this syndrome.[33,35,41–44]

Rationale Basis for Therapy

It is clear from the above that the goal in treating portal hypertension should be to bring back the portal pressure to normal or near normal levels. Many studies have established that when portal pressure is decreased substantially (by more than 20% of baseline or to values below 12 mm Hg), the risk of portal hypertensive complications is markedly decreased and survival is improved.[43] In low-risk situations, such as formation of varices decreases in HVPG as modest as 10% of baseline have been shown to be associated with an improved outcome.[33,42,45]

Reduction of Portal Pressure

Generally speaking, decreasing portal pressure is the kingdom of drug therapy, while normalization of portal pressure is only possible at present by derivative procedures such as transjugular intrahepatic portosystemic shunt or by liver transplantation.

The traditional target for drug therapy has been the hyperkinetic circulation and increased portal venous inflow. Recently, there has been a great effort toward decreasing intrahepatic and portal-collateral resistance pharmacologically, by means of vasodilators, or by enhancing deficient endogenous relaxing factors. In addition, in the next future, treatments will target fibrogenesis, either directly (antifibrotic agents) or indirectly (treatments targeting the cause of the liver disease).[2,36,46]

Symptomatic Treatments

Symptomatic treatments for portal hypertension have been used for many years; these include esophageal tamponade for bleeding varices, endoscopic treatments for esophageal varices, and paracentesis for ascites. These are effective short-term, but recurrence is quite common as the portal pressure is not decreased by treatment.[47,48]

What is Still Needed?

New Evaluation Tools

The most useful and reliable method to evaluate portal hypertensive patients is the measurement of HVPG. Although this is a simple, safe, and quick procedure, it requires specifically trained personnel, a day-hospital admission and its cost is not negligible (direct cost of about 900$)[36] (Figs. 4, 5). Therefore, it would be desirable that less demanding methods were available. Unfortunately, none of the noninvasive methods proposed so far is good enough to substitute for the measurements of HVPG, which remain as the gold standard for clinical evaluation and research.[49]

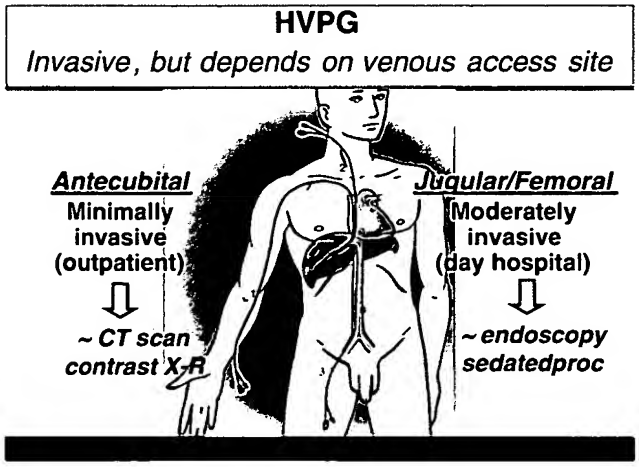

FIGURE 5. Measurement of HVPG is a key tool for the evaluation and management of patients with cirrhosis. Its invasiveness depends on the venous access site.

TABLE 2. Reduction of HVPG Achieved With Different Vasoactive and Drug Combinations

	Mean reduction in HVPG	% Responders*
Non-selective b-bl	10-13.4 %	33-36 %
ISMN	7.5 %	-
b-blocker + ISMN	19-21.4 %	42-51 %
Clonidine	15.4 %	-
Propr + Prazosin	24.2 %	82 %
Carvedilol	19 %	58 %
Losartan	2 %	-
Ideal Drug	25%	90 %*

* Decrease in HVPG to 12 mmHg or below or > 20% of baseline

Better Therapeutic Agents

The vasoactive drugs used at present are still far from ideal. For instance, nonselective β-blockers have contraindications in about 15% to 20% of patients, are poorly tolerated by 18%, and despite its administration, the degree of protection afforded (relative risk reduction) is less than 50%. Probably, this is due in part to the fact that in many patients the reduction of portal pressure achieved with nonselective β-blockers is insufficient. Therefore, there is room for new agents and combinations. These are summarized in Table 2.

Better Prognostic Models and Adjusting Treatments to Patients' Risk

Ideally before starting therapy, we should be able to have an accurate idea of what the patient's risk is. For that purpose, we need much better prognostic models than the available ones (NIEC score, Child-Pugh score, MELD score...). Probably, any new model shall take into consideration portal pressure (or a surrogate, if available), endoscopic findings, age, etiology of the liver disease, on top of the usual markers of disease severity, which are only of value in very advanced stages of cirrhosis. The next logical step would be to adapt the treatment to the patients' risk. It is quite surprising that at present, despite the fact that we do have some robust risk predictors (albeit admittedly not perfect), all patients receive almost identical therapy ab initio, and "intensification" of treatment is only performed after failure of initial therapy.

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Splanchnic and Systemic Vasodilation

The Experimental Models

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Abstract: Experimental models are a sine qua non condition for unraveling the specific components and mechanisms contributing to vascular dysfunction and arterial vasodilation in portal hypertension. Moreover, a careful selection of the type of animal model, vascular bed, and methodology is crucial for any investigation of this issue. In this review, some critical aspects related to experimental models in portal hypertension and the techniques applied are highlighted. In addition, a detailed summary of the mechanisms of arterial vasodilation in portal hypertension is presented. First, humoral and endothelial vasodilators, predominantly nitric oxide but also carbon monoxide and endothelium-derived hyperpolarizing factor, and others are discussed. Second, time course and potential stimuli triggering and/or perpetuating splanchnic vasodilation are delineated. Finally, a brief general overview of vascular smooth muscle signaling sets the stage for a discussion on cotransmission, receptor desensitization, and the observed impairment in vasoconstrictor-induced smooth muscle contraction in the splanchnic and systemic circulation during portal hypertension.

Key Words: portal hypertension, arterial vasodilation, nitric oxide, smooth muscle contraction

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Arterial vasodilation represents the pathophysiologic hallmark in the development of the hyperdynamic circulatory syndrome (HCS) in portal hypertension.[1] This arterial vasodilation occurs early on: it is most predominantly seen in the splanchnic circulation—explaining the tremendous effort to unravel the mechanisms involved—mediating this vascular dysfunction during portal hypertension. In fact, most of the currently available therapeutic agents used for treatment of portal hypertension act by counteracting the vasodilation and thus lowering portal venous inflow.[2,3] In general, blood flow and blood pressure are determined by an integration of reflex, humoral, and local vascular control mechanisms. To dissect and study each of these components with respect to its contribution to arterial vasodilation and increases in blood flow observed in portal hypertension, experimental methods and study designs need to be selected carefully.

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ARTERIAL VASODILATION AND THE HYPERDYNAMIC CIRCULATION

Arterial vasodilation in the splanchnic circulation is the triggering event, which by causing a relative arterial hypovolemia leads to the stimulation of cardiopulmonary volume receptors (atrium, right ventricle, and pulmonary circulation) and arterial baroreceptors (carotic sinus, aortic arch, and juxtaglomerular region of the kidney). It thus activates the sympathetic nervous system (SNS) and the renin-angiotensin-aldosteron-system, as well as the hypothalamic release of arginin-vasopressin (antidiuretic hormone). Mediators of those systems consecutively lead to sodium and water retention and, thus, plasma volume expansion. These compensatory mechanisms aim at normalizing the "effective arterial blood volume" (EABV) within the central circulation (heart, pulmonary, and thoracic aorta) or the so-called central arterial blood volume. The EABV in fact, represents the smallest fluid compartment in the body, enabling "fine-tuning" of volume regulation because, even small changes are sensed by the receptors stated above. In decompensated cirrhosis, EABV remains reduced despite plasma volume expansion because much of the volume is used for filling up the dilated splanchnic circulation, leading to splanchnic pooling.

Plasma volume expansion has been recognized in portal hypertension for many years.[4] In conditions of constant peripheral vascular resistance, an increase in the circulatory blood volume—and thus in the mean circulatory filling pressure—results in increased venous return and cardiac output.[5] However, acute expansion of blood volume leads to stress relaxation of the vasculature and cardiac output after the initial increase returns to normal. This demonstrates that blood volume expansion alone is not sufficient in itself to maintain a hyperkinetic circulatory state. It is the combination of arterial vasodilation (decreased cardiac afterload) and blood volume expansion (increased venous return to the heart) that produces the optimal conditions for maintaining the HCS in portal hypertension.

Although high portal venous inflow per se is not sufficient to induce portal hypertension in the face of a

healthy and highly compliant liver, it is a major contributing factor for the maintenance and aggravation of portal hypertension in conditions of increased resistance to the portal outflow.[6] Increased portal venous blood flow in the face of increased intrahepatic vascular resistance—hence, diminished portal venous perfusion of the liver—can only take place owing to the development of portosystemic collaterals draining portal venous blood into the systemic circulation. Vascular resistance of those collaterals is lower than intrahepatic vascular resistance; however, it is persistently higher than the resistance to flow that a healthy liver displays. Collateralization thus only partially decompresses the portal system, and portal hypertension persists.

The clinical consequences of generalized arterial vasodilation and the HCS in portal hypertension are not restricted to the hepatosplanchnic circulation. Intrapulmonary vasodilation causes impaired arterial gas exchange, leading to arterial hypoxemia in the absence of detectable cardiorespiratory diseases: the so-called hepatopulmonary syndrome (HPS).[7] Similarly, vasodilation of the cerebral vasculature has been proposed to increase the capillary surface and thus, to facilitate the diffusion of noxious gut-derived compounds such as ammonia, which can contribute to the development of hepatic coma.[8] A strong correlation between cerebral blood flow, brain swelling, and outcome and depth of coma[9] support the hypothesis that the hyperdynamic cerebral circulation might contribute to brain swelling, intracranial hypertension, and hepatic coma.

THE OPTIMAL EXPERIMENTAL MODEL AND APPROACH?

Animal Models

Most of the knowledge on the pathogenesis of arterial vasodilation in portal hypertension gained over the last decades has mainly been derived from animal models. Portal hypertension is not only the consequence of an increased resistance to portal blood flow, but also of an increase in portal inflow. This concept could not, in fact, be evidenced until the development of an adequate methodology to conduct detailed hemodynamic studies in animal models of portal hypertension.[10,11] However, the experimental models available differ largely in time course and severity of portal hypertension, as well as in the degree of liver injury and failure.[12] Moreover, each animal model has to be judged with respect to reproducibility, specificity, and feasibility to ensure an appropriate degree of quality. This also is a basal necessity to obtain valid results that can most likely sustain the challenge of testing in comparable human pathologic conditions.

For the study of splanchnic vasodilatation and the development of the HCS in general, prehepatic as well as intrahepatic animal models of portal hypertension can be used. However, due to the pioneering work of Chojkier and Groszmann in establishing the *partial portal-vein ligated (PVL-) rat model*,[13,14] a highly reproducible, cheap, and easy to perform model became available. This model is created after midline laparotomy by a simple ligature around a blunt-tipped needle (20G) lying along the portal vein. Subsequent removal of the needle yields a calibrated stenosis of the portal vein.

The PVL model represents the ideal experimental model for dissecting the different components contributing to the development and maintenance of the HCS. Moreover, most importantly, the PVL model enables a detailed investigation of the initiating triggers that start off the sequence of events finally leading to the HCS. For instance, 2 independent studies, although using different methods, have found a basically identical sequence of circulatory changes in the splanchnic vasculature after portal-vein ligation in rats.[15,16] This demonstrates that the splanchnic hemodynamic characteristics of this model are exactly predictable and extraordinarily homogenous. One week after PVL surgery, the rats present the complete portal-hypertensive syndrome with HCS and portal-systemic shunting. Portal-systemic shunting is already detectable at 2 days after portal-vein ligation. The percentage of portal-systemic shunting, that is, the amount of portal blood inflow diverted to collaterals, approaches 100% after 1 week.[16] However, the main drawback of this model is that portal hypertension develops acutely and the degree of portal hypertension is maximal at 24 hours, degrading afterward. This clearly contrasts with the majority of clinical situations in daily practice. Nonetheless, by producing less severe portal-vein stenosis (using needles of greater caliber), models without splanchnic arterial reflex vasoconstriction have recently been developed.[12,17,18] In fact, this situation is very similar to that which occurs initially in chronic liver disease; thus, it enables the investigation of the hemodynamic alterations and the triggering molecular signals for the development of the HCS, comparable with the early stages of chronic liver disease.

Nonetheless, *other models* mimicking human liver disease have also been used successfully. They were pioneered in a similar manner by Groszmann et al, for example, experimental schistosomiasis.[19–21] An important feature of this presinusoidal intrahepatic model is that portal hypertension develops progressively. However, now this model is rarely used. Finally, widely used intrahepatic sinusoidal models of portal hypertension include common bile duct ligation (CBDL) and carbon tetrachloride or thioacetamide-induced liver cirrhosis [carbon tetrachloride (CCL_4) and thioacetamide, respectively].[12,22,23] The CBDL model aims to represent secondary biliary cirrhosis but encounters several limitations owing to its quite aggressive course. In fact, mortality is high, architectural changes typical of cirrhosis are only rarely observed, and portal hypertension is at least partly due to mechanical (pre)sinusoidal congestion. Nonetheless, the CBDL rat is the only established model that reproduces the physiologic features of human HPS.[24] The question of why other rodent models of cirrhosis and/or portal hypertension do not develop HPS remains as yet enigmatic and unsolved. Administration of CCL_4

causes liver injury via cytochrome P450-mediated production of the radical CCL_3 and the associated toxic hepatocellular damage. Phenobarbital is usually added to drinking water (0.3 g/L) to increase the metabolization of CCL_4 and the consequent production of toxic CCL_3, enhancing the yield of liver cirrhosis. Hemodynamic studies are performed 1 week after the cessation of CCL_4 and phenobarbital administration. Various routes of administration of CCL_4, namely inhalation, ingestion by gavage, or intraperitoneal injection, are used. Twelve to 16 weeks after CCL_4 administration, the rats develop micronodular cirrhosis, portal hypertension, portal-systemic shunting (30% to 60%), and HCS. If the CCL_4 administration is maintained for 12 to 20 weeks, most of the animals will develop ascites. However, the time course and the rate and severity of cirrhosis vary among individual animals owing to the differing susceptibilities of the rats to CCL_4-induced hepatocellular damage. Therefore, individualization of the dose according to the weight gain/loss of the animal (in response to the previous dose) has been suggested.[25] Thioacetamide is a toxin affecting the perivenular and the periportal areas; it can be administered in drinking water or by intraperitoneal injection, with the latter approach leading to more consistent results. This model develops macronodular cirrhosis in about 12 weeks. However, even longer periods might be required to induce overt HCS.[26]

Experimental Design and Methods

Regarding in vivo studies, focusing on the hemodynamic changes in portal hypertension and the invasive monitoring of the arterial and portal pressure measurements of cardiac output and regional blood flow are the most important features of an ideal experimental design. For the assessment of cardiac output in animals, different methods have been used: Fick's method, several indicator dilution techniques, thermal dilution, radioisotope dilution, electromagnetic flowmetry, and impedance cardiography. Despite potential overestimation in small animals, the thermodye technique has been used most widely in animal models of portal hypertension owing to its high degree of reproducibility, easy performance, and minimal discomfort to the animal.[17,27–29] In respect of regional blood flow, the use of colored or radioactive microspheres is very common. Using the reference sample method and relying on well established performance criteria,[30] this approach is low in relative error and variability.[31] Moreover, direct evaluation of blood flow velocity (eg, Doppler flowmetry) or even volumetric blood flow (eg, transit-time ultrasound flowmetry) has been achieved by miniaturizing the corresponding flow probes. In vivo assessment of blood flow in different vessels such as the superior mesenteric artery (SMA), the portal vein, or even the portosystemic collaterals in experimental portal hypertension thus became available. This clearly enriched the experimental armentarium available for investigating hemodynamic alterations during portal hypertension. In fact, these techniques permit repetitive, noninvasive measurements under near physiologic conditions without anesthesia, restraint, surgical stress, or manipulation.[32] In this context, it is important to stress that any technique applied for hemodynamic measurements in anesthetized animals depends on the careful use and selection of anesthetic drugs with respect to variability, reproducibility, and accuracy. To minimize their influence on hemodynamics, for example, particularly on the liver and splanchnic blood flow as well as on the cardiac output, neuromuscular blocking agents and halothan should be avoided.[33,34]

Regarding the in vitro studies delineating the pathogenetic mechanisms leading to arterial vasodilation in portal hypertension, it is expeditious to consider several key aspects related to the choice of vessel to study and the optimal methodology to use. First, the main site of the changes in vascular resistance is the splanchnic arterial vasculature. Therefore, the SMA—the largest single branch of the abdominal aorta—supplying the entire small intestine, the proximal portions of the colon, and the pancreas represents the ideal vessel to be studied in portal hypertension. In contrast, it is not suitable to use aortae because they constitute conduit vessels that do not contribute to the regulation of systemic and/or peripheral vascular resistance in vivo. Second, the type of isolated vessel preparation needs attention. The use of simple vessel strips or rings per se is limited in its applicability because the mechanisms of blood-flow control as well as the individual contributions to vascular resistance change along the vascular tree (from large to small arteries, arterioles, and capillaries). In fact, the major site of resistance resides in the microvessels (true resistance vessels with a size range of about 10 to 150 μm), which are much smaller than the blood vessels commonly studied by established in vitro methods. Therefore, most of the data presented in the following section have been obtained by using the McGregor[35] preparation of the mesenteric vascular bed. This approach has the unequivocal advantage of using the whole arterial tree in its natural geometrical arrangement, thereby including every site of vascular resistance. Moreover, by being perfused in vitro with Krebs solution, this method is free from any influence from nerval, hormonal, or cellular interactions. Third, caution is necessary in extrapolating data obtained in resting vascular smooth muscle (VSM) to find out how it relates to the mechanisms by which contracted smooth muscle relaxes. Therefore, stable preconstriction levels, ideally at EC_{80} concentrations, are required to investigate vasodilative capacity of various vasodilators. In this respect, also at the same preconstriction level, the vasodilative response to receptor-dependent and nonreceptor-dependent vasodilators will differ, depending on the type of vasoconstrictor used for preconstriction.

MECHANISMS OF ARTERIAL VASODILATION IN PORTAL HYPERTENSION

The balance between the net effects of the vasoconstrictors and the vasodilators acting on the VSM

determines the level of vascular tone. The observed reduction in vascular tone in the splanchnic circulation in portal hypertension is caused by both (a) increased vasorelaxation due to the enhanced release and levels of vasodilative mediators and (b) vascular hyporeactivity to vasoconstrictors and impaired signaling in VSM (Fig. 1).

Enhanced Release and Levels of Vasodilative Mediators

Early cross-perfusion studies between portal-hypertensive and normal rats have shown an increase in flow and a decrease in vascular resistance in the splanchnic and systemic circulations of the normal animal.[36,37] This clearly indicated the presence of circulating vasodilative substances playing a key role in the development of splanchnic vasodilation during portal hypertension. The failure of a parabiotic model (1 portal-hypertensive, 1 normal) to confirm these findings, however, points toward rapid metabolism of the circulating vasodilator(s) by the liver of the normal animal.[38] This failure might also indicate that humoral vasodilators are less important than endothelium-dependent vasodilators, which are usually entirely metabolized at the site of production. Indeed, much attention has focused in recent years on the release and hemodynamic effect of nitric oxide (NO), one of the most potent vasodilators being synthesized by 3 different isoforms of nitric oxide synthases (NOS). The following sections will thus focus first on circulating hormonal vasodilators and then on endothelium-dependent vasodilators with particular focus on NO.

Humoral Vasodilators

A variety of gut peptides have been proposed as the circulating vasodilatory agents mediating the development of the HCS, including glucagon, adrenomedullin, atrial natriuretic peptides, etc. Several early studies in experimental and human portal hypertension revealed elevated glucagon serum levels.[39,40] The infusion of glucagon in normal animals to achieve the levels observed in portal-hypertensive animals is, in fact, associated with a significant reduction in splanchnic vascular resistance,[36] and the infusion of glucagon-specific antiserum results in a significant reduction in portal venous inflow.[41] The infusion of pharmacologic doses of somatostatin and its synthetic analog octreotide, which inhibit glucagon release, produces vasoconstriction of the splanchnic circulation.[42] The simultaneous infusion of somatostatin and glucagon prevents the decrease in portal venous inflow and portal pressure observed in portal-hypertensive animals receiving somatostatin alone.[43] These results suggest that the somatostatin-induced vasoconstriction is, at least in part, due to the inhibition of the glucagon vasodilatory activity. Somatostatin inhibits the release of several other vasodilator peptides such as the vasoactive intestinal peptide, substance P, calcitonin-gene-related peptide (CGRP), and insulin (all known to be more or less increased during portal hypertension). Therefore, the effect of somatostatin on the hyperdynamic circulation might be mediated by other peptides in addition to glucagon. Increased glucagon levels cannot be solely responsible for the HCS. The infusion of glucagon antiserum is not accompanied by an increase in blood pressure or by a significant reduction in portal pressure.[41] Another study has failed to show a correlation between the magnitude of vasodilation and circulating levels of glucagons.[40] In addition, somatostatin decreases but does not normalize portal venous inflow[42] and has only a mild effect during fasting states.[44]

Circulating levels of adrenomedullin, a potent vasodilative neuropeptide, are also increased in liver cirrhosis, and they correlate with hemodynamic abnormalities and the activation of vasoconstrictor systems.[45] Concentrations of adrenomedullin comparable with those found in ascitic cirrhotic patients have a vasodilator effect in the rat mesenteric circulation and induce arterial hypotension.[46] It has been suggested that, at least in part, this adrenomedullin-mediated vasodilation can occur through the production of NO.[47] Also, atrial-natriuretic peptide levels tend to increase in the advanced stages of liver cirrhosis with ascites.[48] Atrial-natriuretic peptide is both a direct vasorelaxant and an antagonist of the vasoconstrictive effects of angiotensin II. Other endogenous humoral vasodilators including adenosine, histamine, and bile salts have been investigated, but they have not been found to play a significant role in the arterial vasodilation in portal hypertension.[49,50]

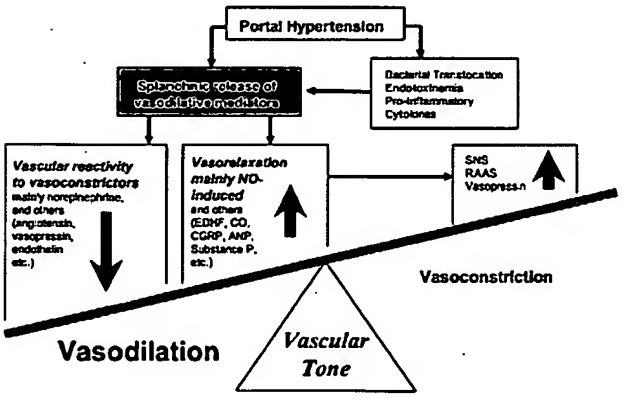

FIGURE 1. Mechanisms contributing to arterial vasodilation in portal hypertension. The reduction in vascular reactivity to vasoconstrictors and the increase in vasodilative mediators acting on the vascular smooth muscle cell together cause net arterial vasodilation despite compensatory stimulation of the SNS, RAAS, and many other vasoconstrictive systems. This imbalance is further aggravated by the occurrence of BT and endotoxinemia, which have been proposed to be accompanied by enhanced gut-derived release of proinflammatory cytokines.

Endothelium-dependent Vasodilators

NO and NOS isoforms (for further details and enzyme regulation see chapter Y. Iwakiri)

Three isoforms of NOS have been cloned and characterized in detail so far (reviewed in Ref. 51). Two

distinct but related NOS isoforms, one synthesized by endothelial cells [endothelial NO-synthase (eNOS)] and the other by neuronal cells [neuronal NO-synthase (nNOS)], are expressed constitutively. eNOS releases NO for short periods of time in response to several endogenous and exogenous stimulants, including physical stimuli such as shear stress. Shear stress is the key mechanical force induced by blood flow that stimulates vasodilation via direct and immediate enhancement of eNOS-derived NO release.[52] eNOS is stimulated further by receptor-dependent agonists such as catecholamines (particularly via α_1 adrenoreceptors), endothelin (via ET_B receptors), angiotensin (via AT_1 receptors), muscarinic agonists (eg, acetylcholine) etc. Depolarization of nerve endings activates nNOS. The presence of such nNOS-containing perivascular nerves has been demonstrated in numerous vascular beds and in multiple species.[53,54] Functional studies underline the importance of these adventitial nNOS-immunoreactive fibers for the regulation of vascular tone and for mediating the neurogenic or the so-called nitrergic vasodilation by releasing NO.[55] The inducible NO-synthase (iNOS) is synthesized de novo in VSM cells, macrophages, hepatic stellate cells, hepatocytes, and many others after induction by lipopolysaccharides (LPS) and/or proinflammatory cytokines. Once expressed, iNOS synthesizes large amounts of NO for extensive periods of time, independent of any hemodynamic or mechanical stimuli.

In vivo evidence for the role of NO for arterial vasodilation in portal hypertension. Several in vivo studies in experimental portal hypertension underline the importance of NO for arterial vasodilation and the associated hemodynamic abnormalities stated above. Cirrhotic rats with ascites exhibit increased sensitivity to the pressor effect of NO inhibition, the increment in blood pressure in response to L-NNA being greater than in control animals.[56] In prehepatic portal-hypertensive rats, systemic vascular hyporesponsiveness to vasoconstrictors, demonstrated as a diminished increase in systemic vascular resistance in response to methoxamine compared with sham rats, is reversed by the short-term administration of L-NNA.[57] Moreover, acute administration of a nonspecific NO-synthesis inhibitor in vivo has been reported to almost completely normalize splanchnic hemodynamics in CCL_4-induced liver cirrhosis or prehepatic portal hypertension.[58,59] In addition, chronic inhibition of NO synthesis in PVL rats ameliorates portal-systemic shunting by increasing splanchnic arterial vascular resistance as well as portal collateral resistance and corrects peripheral vasodilation and systemic capillary hypotension.[28,60] Similarly, chronic inactivation of overproduced NO, by increasing blood hemoglobin—binding and depleting NO—attenuates splanchnic vasodilatation in portal hypertension.[61] Finally, titrating doses of L-nitroarginine methylester, a nonspecific NO inhibitor to reduce aortic cyclic guanosine monophosphate (cGMP) concentrations to normal, indicating normalization of NO production, in cirrhotic rats with ascites was associated with the normalization of arterial pressure, cardiac index, and total vascular resistance.[62] However, mice with the targeted deletion of eNOS (as well as both eNOS and iNOS) still develop the HCS, similar to wild-type animals after partial portal-vein ligation.[29] In the absence of compensatory up-regulation of nNOS,[63] this strongly suggests the existence of other vasodilator molecules being involved in the pathophysiology of arterial vasodilation and the development of HCS in portal hypertension.

In vitro evidence for the role and source of NO for arterial vasodilation in portal hypertension: Splanchnic circulation. Vascular hyporesponsiveness in the splanchnic circulation is observed for a diversity of vasoconstrictors; of these, mainly norepinephrine, angiotensin, methoxamine, vasopressin, and endothelin have been studied.[64–69] This impaired vasoconstrictor response is thus not dependent on the class of receptor. Moreover, nonreceptor-mediated vasoconstriction (e.g. by potassium-chloride or periarterial nerve stimulation) is reduced in portal-hypertensive animals.[70,71] In general, responsiveness is reduced owing to the amelioration of vascular contractility, whereas vascular sensitivity is maintained during portal hypertension. This general vasoconstrictor defect has been mainly localized to the endothelium because the removal of the endothelium almost completely corrects this in vitro vascular hyporeactivity.[72] The main endothelium-derived vasodilator responsible has been shown to be NO as inhibition of NO biosynthesis restores vascular reactivity to (near) normal levels.[66,67,70]

Enhanced endothelial NO release has directly been shown by monitoring NO*x* concentrations (via chemiluminescence) in the perfusate that drains from the in vitro perfused mesenteric vasculature in response to vasoconstrictors or increases in flow and, thus, shear stress.[73,74] Moreover, at each level of flow-induced shear stress (as was estimated by the changes in perfusion pressure and flow rate), PVL rats produced more endothelial NO than normal controls. Similarly, in response to α_1-adrenergic stimulation, despite the lower pressure response and the consequent shear stress, increased NO*x* concentrations in the perfusate outflow were observed in PVL animals.[75] These results demonstrate that shear stress-induced release of NO from the superior mesenteric arterial endothelium is increased in portal-hypertensive animals. Moreover, the mesenteric arteries of PVL rats contain several times more eNOS protein than similar vessels from normal rats, and the concentration of mRNA is similarly increased.[74,76] A similar up-regulation of eNOS can also be demonstrated in cirrhotic rats, as evidenced by the increased eNOS protein levels and enhanced Ca^{2+} dependent NOS activity.[77–79] In detail, enhanced eNOS activity was observed in the SMA as well as in the mesenteric arterial branches distal to the SMA, the microcirculation where vascular resistance is determined. This eNOS up-regulation mediates the observed increases in responses to endothelium-dependent vasodilator substances such as acetylcholine, which are prevented by the inhibition of NO synthesis.[80,81] In addition to this enhanced eNOS machinery also enhanced

nNOS expression and nNOS function has been reported in the splanchnic vascular bed of PVL rats.[82–84] In fact, increased nitrergic vasorelaxation in the mesenteric arteries of portal-hypertensive rats, which were being corrected by inhibition of NO-synthesis, has been demonstrated. These results demonstrate that eNOS-derived and nNOS-derived NO is vastly increased in portal-hypertensive animals in the mesenteric circulation and that it plays a major role in modulating the vascular tone and responsiveness to vasoconstrictors. In contrast, multiple investigators have so far failed to prove convincingly the need for iNOS up-regulation and/or the involvement of iNOS-dependent NO production in arterial vasodilation in portal hypertension.[79,85]

Systemic circulation. Although vascular NO overproduction is predominantly of splanchnic origin[86] and although the splanchnic circulation is thus the main site of reduction in vascular resistance, central arterial and peripheral cutaneous and muscular arterial vasodilation can also contribute.[1,87,88] Comparable with the splanchnic vasculature, aortae of portal-hypertensive rats exhibit an impaired responsiveness to vasoconstrictors, which is mediated largely by endothelium-derived NO[89–91] and exhibits resistance to the preferential inhibition of iNOS.[89,90] Moreover, vasodilator response to eNOS agonists is enhanced in the aortic rings of portal-hypertensive rats.[80,91] Finally, systemic nNOS inhibition in cirrhotic rats in vivo for 1 week using the selective nNOS inhibitor 7-NI resulted in the normalization of systemic vascular resistance, mean arterial pressure, and cardiac index.[92] These data also underscore the eNOS and nNOS up-regulation and the associated NO overproduction in the systemic circulation mediating arterial vasodilation. In addition, increased pulmonary eNOS-derived NO synthesis seems to be a key component in the pathogenesis of the HPS in CBDL rats.[93] In detail, endothelin-1–triggered ET_B-receptor-dependent pulmonary eNOS up-regulation has been evidenced during HPS in CBDL rats, and selective ET_B-receptor inhibition improves HPS.[94] Moreover, the accumulation of intravascular macrophages and the associated iNOS-derived NO-production might also contribute to the severity of the experimental HPS.[95]

Time course of vascular NO overproduction in portal hypertension. It is well known that chronic increases in blood flow induce the up-regulation of eNOS. Increased eNOS-derived NO production can thus be easily explained as the normal adaptation of the endothelium to the splanchnic high flow state and to the presence of enhanced shear stress in portal hypertension. In accordance with this hypothesis, more pronounced vascular NO overproduction is observed in cirrhotic rats with ascites compared with those without ascites.[78,96] However, eNOS up-regulation in the mesenteric vasculature precedes the development of the hyperdynamic splanchnic circulation in portal hypertension.[97] This has been evidenced by investigating PVL animals early after portal-vein ligation. The sequence of events after PVL is characterized by vasodilation in nonsplanchnic vasculature and vasoconstriction with concomitantly decreased

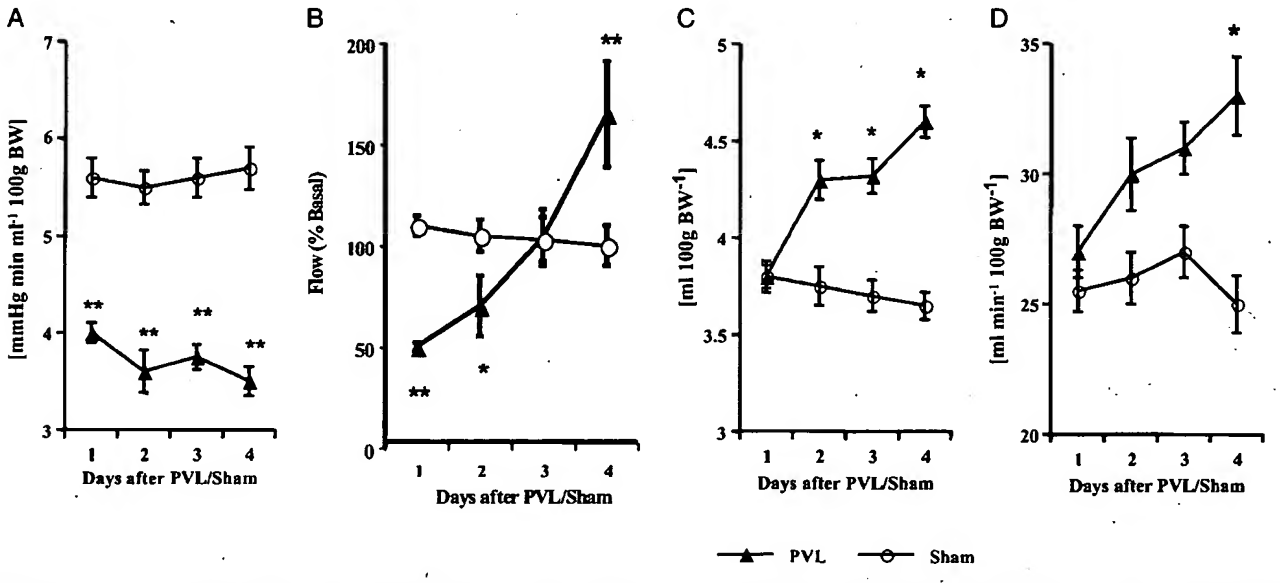

FIGURE 2. Time course of splanchnic blood flow and HCS after portal-vein ligation. Peripheral vasodilation (in the nonsplanchnic circulatory bed) has already occurred on day 1 after portal-vein ligation (A: total peripheral resistance) and is the first hemodynamic event in the development of the HCS. As can be appreciated, shortly after surgery mesenteric vasoconstriction occurs (B: SMA-blood flow), representing a reflex mechanism in response to the portal-outflow block. On day 3, however, splanchnic blood flow is normalized in comparison with sham animals. By day 4, the splanchnic circulation becomes hyperdynamic. Cardiac index and regional blood flow increase in parallel with plasma volume expansion. On day 4, maximum values of plasma volume (C) coincide with fully developed HCS, as shown by a significant elevation in the cardiac index (D) and the regional blood flows. *$P<0.05$, **$P<0.01$ versus Sham animals. Reprinted from *Hepatology.* 1992;15:323–328.[15]

blood flow in the mesenteric circulation, secondary to a myogenic reflex induced by the acute increase in portal pressure.[15] It is not until day 4 or 5 after portal-vein ligation that the circulation becomes hyperdynamic[16] (Fig. 2). In detail, on day 3 after portal-vein ligation, animals exhibit SMA blood flow and total portal venous inflow not different from the flows in sham rats.[15,16] Already under such normodynamic conditions, increased eNOS-derived NO production by the mesenteric vasculature has been observed (Fig. 3).[97] In detail, the NO*x* production rate of the mesenteric vasculature, in response to the incremental increases in flow rate and the α_1-adrenergic stimulation and associated shear stress, is significantly higher in PVL rats than in sham animals. Concomitantly, the pressure response to the changes in flow rates as well as the α_1-adrenergic stimulation are reduced in PVL animals; however, the blockade of NO synthesis abolished this difference in vascular responsiveness. Moreover, higher eNOS protein levels and eNOS enzymatic activity in mesenteric arteries could be demonstrated by day 3 after PVL.[97] Therefore, eNOS-mediated vascular NO overproduction is not secondary to chronic increases in flow and shear stress and in fact, might play a primary role in the pathogenesis of the hyperdynamic circulation in portal hypertension.

One of the recently identified positive modulators of eNOS is the chaperone heat shock protein 90 (HSP90), which binds to eNOS in response to growth factors, increasing its catalytic activity.[98] In fact, enhanced HSP90 signaling seems to contribute to NO-dependent vascular hyporeactivity in portal-hypertensive splanchnic vasculature.[99] Likewise, the well known importance of Akt-mediated phosphorylation of eNOS in modulating eNOS activity has been substantiated in superior mesenteric arteries in early portal hypertension.[100] In detail, significantly increased Akt phosphorylation and associated increases in phospho-eNOS, and thus catalytic enzymatic activity in SMA, could be evidenced as early as 1 day after 20G portal-vein ligation.[27] At this time point, no changes in eNOS expression could be observed; therefore, it seems that the up-regulation of eNOS enzyme activity, mediated by the enhanced phosphorylation of eNOS by Akt, might at least in part represent the initial event leading toward NO overproduction and vasodilation in portal hypertension (for more details see chapter Y. Iwakiri). However, in our view, none of these intermediary factors are the primary reason for the increase in eNOS in the splanchnic and systemic circulation in cirrhosis.

Stimulus for vascular NO overproduction and the mechanisms involved. Two recent elegant investigations might further delineate the time course of splanchnic

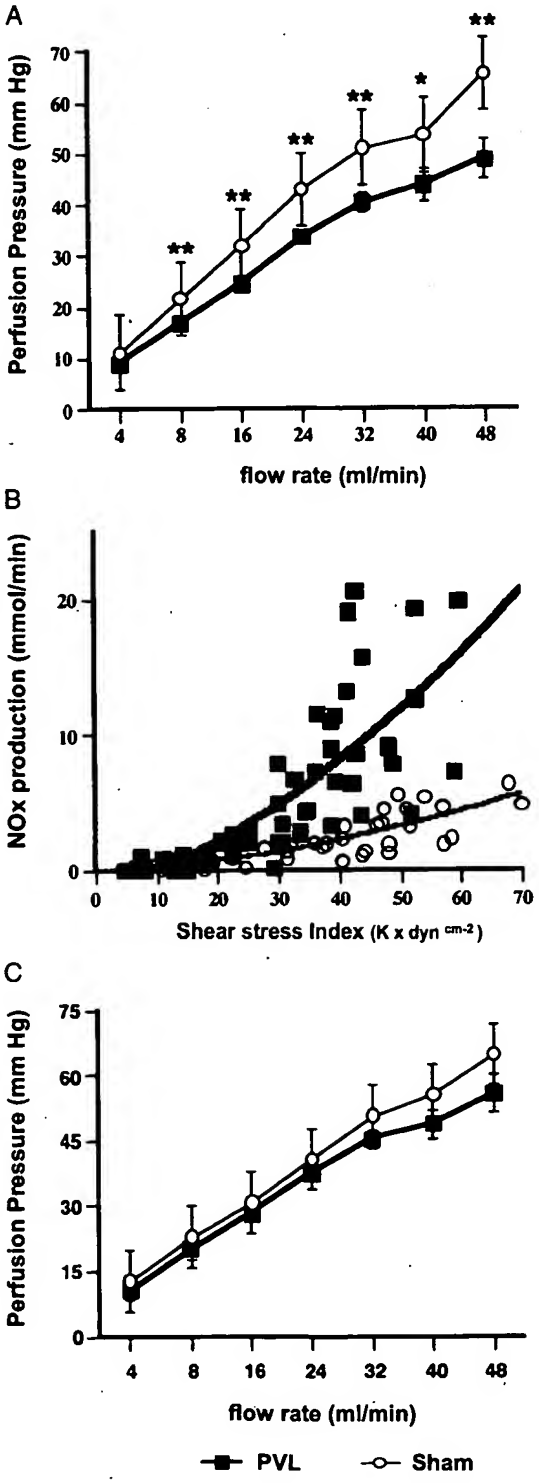

FIGURE 3. Shear stress-mediated NO release in the mesenteric arteries of PVL rats under normodynamic conditions (day 3 after PVL). In-vitro perfused mesenteric vascular bed of PVL and sham rats. Pressure response to increases in flow rates was significantly reduced in PVL rats (A) **P*<0.05; ***P*<0.01 versus Sham rats. In parallel, the slope of NO_x production induced by shear stress was markedly increased in vessels from PVL rats compared with sham animals (B) (*P*<0.001; analysis of variance). After NO inhibition (C), pressure response to changes in flow rates were no more significantly different between the study groups evidencing NO dependency of observed hyporesponsiveness in PVL rats. Reprinted from *Am J Physiol.* 1999;276(4 Pt 1):G1043–G1051.

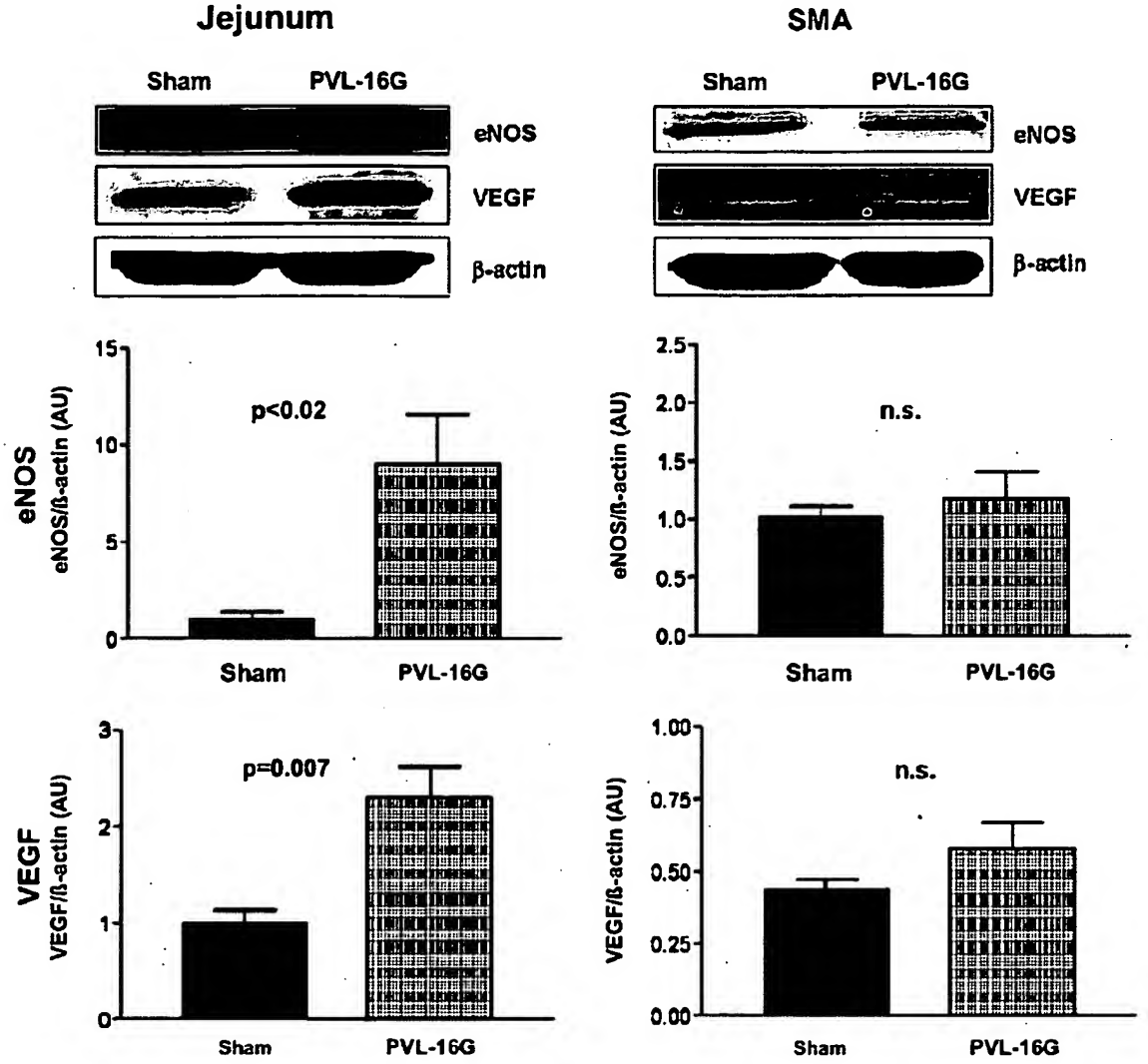

FIGURE 4. eNOS up-regulation at the intestinal mucosa and mesenteric arteries at day 1 after PVL 16G. Expressions of eNOS and VEGF are up-regulated in the jejunal microcirculation, but not in the SMA, 24 hours after PVL-16G or sham surgery. AU indicates arbitrary units. Reprinted from *Am J Physiol.* 2006;290:G980–G987.

eNOS up-regulation in prehepatic portal hypertension.[17,18] Tsai et al[101] could demonstrate enhanced eNOS activity and NO-mediated vascular hyporesponsiveness in the SMA, as early as 10 hours after the induction of severe portal hypertension (20G stenosis of the portal vein). This eNOS up-regulation seems to be triggered by mesenteric vasoconstriction (and thus, cyclic strain): rats with renal artery ligation exhibiting increased mesenteric resistance (but no portal hypertension) also presented with a similar enhancement in eNOS activity and associated vascular hyporesponsiveness. In contrast, portal hypertension per se in the absence of mesenteric vasoconstriction—created by low-level stenosis of the portal vein (18G diameter)—was not associated with alterations in vascular responsiveness or eNOS activity at the level of the SMA. Therefore, Abraldes et al[17] raised the question of whether other signals located at more sensitive areas such as the intestinal microcirculation might activate splanchnic NO production. In fact, even within 24 hours of creating a lower-level stenosis of the portal vein (16G diameter), activation of eNOS could be evidenced selectively at the intestinal mucosa microvasculature (Fig. 4). Most interestingly, these early changes were observed in the absence of any significant changes in mesenteric blood flow or resistance; thus, they clearly precede the development of splanchnic vasodilation. Moreover, such mild increases in portal pressure were found to be associated with a marked up-regulation of vascular endothelial growth factor (VEGF) expression predominantly at the mucosa in highly vascularized areas. Finally, VEGF-receptor-2 blockade in vivo markedly attenuated eNOS up-regulation at the mucosal microvasculature in 16G-PVL rats. Considering the well known activation and up-regulation of eNOS by VEGF,[102] these

data suggest that the already present mild increases in portal pressure, which might be similar to what occurs in early cirrhosis, trigger the production of VEGF and an increase in eNOS expression in the intestinal microcirculation. These phenomena most likely represent one of the initiating molecular signals triggering the cascade of events leading to the HCS.

Moreover, the tumor necrosis factor (TNF) has been reported to enhance endothelial NO synthesis by increasing eNOS activity[103] and has been implicated in the development of the HCS. Indeed, TNF inhibition ameliorates the HCS in PVL rats.[104,105] TNF is known to be released from the gut in conditions of bacterial translocation (BT); BT has been reported to be increased on day 2 after induction of PVL[106] but is most likely present even in the very early phase after PVL. BT has also been demonstrated to increase eNOS-derived NO synthesis in the mesenteric arteries in cirrhosis, leading to an additional impairment in vascular contractility.[79] Correspondingly, cirrhotic rats with BT presented with a lower mean arterial pressure, indicating a more severe arterial vasodilation. Because BT is the earliest step in the development of endotoxinemia and the secondary release of TNF, this might explain earlier reports demonstrating a close correlation between serum levels of endotoxin and NO in liver cirrhosis.[107] However, it remains to be clarified to what extent endotoxins and TNFα contribute to vascular NO overproduction in portal hypertension. There is an essential need to separate the individual etiopathologic factors involved in portal hypertension.

A potential explanation for this "paradoxon" of enhanced NO synthesis in conditions of endotoxinemia and TNF secretion, but absence of iNOS induction in liver cirrhosis, could be tetrahydrobiopterin (BH_4), an essential and rate-limiting cofactor for NO synthesis. Recently, it has been established that in endothelial cells, TNFα and LPS stimulate gene expression and activity of the key enzyme for the regulation of BH_4 biosynthesis—guanosine-triphosphate-cyclohydrolase I (GTPCH-I).[108–111] Considering that BH_4 can directly increase eNOS-derived NO bioavailability,[112,113] TNFα and LPS can directly increase vascular NO synthesis in the absence of any induction of iNOS by up-regulating the production of BH_4.[103,110] In fact, GTPCH-I activity and BH_4 content was found to be increased in the mesenteric vasculature in cirrhotic rats only in conditions of BT.[114] Moreover, both GTPCH-I activity and BH_4 content correlated positively with serum NO*x* levels and mean arterial pressure, indicating a major vasodilatory effect and hemodynamic impact of GTPCH-I up-regulation via the enhancement of vascular NO production. The observed correlations between GTPCH-I activity and BT-associated increases in serum endotoxin and TNF, known to stimulate GTPCH-I, support the hypothesis that in advanced cirrhosis, BT and/or infectious complications aggravate arterial vasodilation via GTPCH-I up-regulation and associated increases in BH_4/NO synthesis (Fig. 5). However, it is difficult to evidence this hypothesis because GTPCH-I is a ubiquitous enzyme and is abundantly

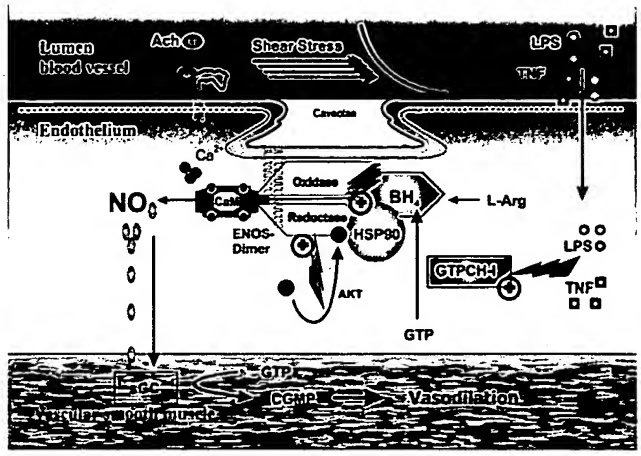

FIGURE 5. Mechanisms of eNOS regulation and eNOS-derived NO synthesis. NOS consist of an oxygenase (heme-containing) domain and a reductase [NADPHcytochrome P450-like and diflavin (FMN, FAD)-containing] domain. Both domains are linked via a regulatory calmodulin-binding linker to form one large polypeptide. Blood flow and associated shear stress or receptor-dependent agonists such as acetylcholine (Ach) stimulate eNOS-derived NO release. Phosphorylation of eNOS by the serine/threonine protein kinase AKT enhances its ability to generate NO. HSP90 facilitates eNOS-dependent NO production. LPS and/or TNF stimulate endothelial GTPCH-I activity and, thus, production of tetrahydrobiopterin (BH_4). BH_4 has profound effects on eNOS dimer structure and serves as electron donor/redox-active cofactor for the N hydroxylation of L-arginine, increasing eNOS activity and eNOS-derived NO synthesis.

expressed, and BH_4, its product, is involved in other enzymatic reactions independent of NOS. Therefore, any inhibition of GTPCH-I in vivo can lead to multiple various effects that are completely independent of its impact on NOS. Moreover, inhibitors for GTPCH-I have been criticized for being nonspecific and for inducing multiple secondary events independent of its effect on BH_4. Intestinal decontamination by antibiotics, nonetheless, demonstrated that increased BH_4 availability, as a result of BT, does indeed contribute to increased eNOS activity in these animals.[115]

In recent years, the role of circulating eNOS-activating hormones in vascular NO overproduction has also been revealed. Increased plasma levels of circulating vasodilators acting via NO/cGMP were demonstrated. Among them, the endocannabinoid system seems to contribute strongly to the development of splanchnic vasodilation, driving the pathogenesis of the HCS.[116] Activation of endothelial CB1 receptors by their endogenous ligands (anandamide, 2-arachidonyl glycerol) causes activation of eNOS. It has been credibly shown that increased endocannabinoid production and up-regulation of CB1 receptors contribute to the increased mesenteric NO production by eNOS in mesenteric vessels from portal-hypertensive rats.[116–118] Other candidates

that potentially contribute to eNOS up-regulation include substance P, adrenomedullin, estrogen, and catecholamines, which have been shown to increase eNOS expression and activity and are all known to be increased in chronic portal hypertension.[119–121]

Finally, it is noteworthy to mention that the increased eNOS-derived vascular NO production persists despite elevated levels of *asymmetric dimethylarginine (ADMA)*, which is an endogenously produced inhibitor of eNOS. Such increased serum levels of ADMA were observed in BDL, but not in PVL, rats[122] and in patients with alcohol-induced liver cirrhosis.[123] Because ADMA contributes to endothelial dysfunction within the intrahepatic microvasculature, but has apparently no effect in extrahepatic vessels, this suggests the different regulation of eNOS by ADMA in different vascular beds. The ADMA-degrading enzyme, dimethylarginine dimethylaminohydrolase, represents a possible candidate that might account for this discrepancy.[122]

Other Contributing Vasodilators

An *endothelium-derived hyperpolarizing factor* (EDHF) has been shown to be a major endothelium-dependent vasodilator in resistance vessels of eNOS-knockout mice.[124,125] In fact, EDHF was first described as an endothelium-dependent relaxant that was resistant to the inhibition of NO and/or cyclooxygenase (COX), but was inhibitable by potassium channel blockers. In experimental portal hypertension, endothelial up-regulation of small-conductance Ca^{2+}-dependent K^+ channels[126] has been reported, contributing to the well known vascular hyporesponsiveness to endogenous vasoconstrictors.[127] The role of EDHF for vascular regulation has been shown to become more prevalent when NO production is inhibited.[128] This latter fact has been attributed to the observation that NO attenuates EDHF release.[129] In other words, hyperpolarizing mechanisms can take over when NO is inhibited—a scenario that might contribute to the observed development of the HCS in eNOS-knockout mice.[29]

Moreover, endothelial vasodilator *prostaglandins* are up-regulated in the absence of eNOS, contributing to flow-induced arterial vasodilation.[130] Indeed, vasodilative prostanoids such as prostaglandine I2 (PGI2) have been reported to contribute to splanchnic vasodilation in experimental portal-hypertensive conditions.[131,132] Both COX-1 and COX-2 are up-regulated in hypocontractile vessels of PVL rats.[131,133] By contrast, in another study, changed activities of enzymes involved in PGI2 synthesis were not found in aortas from PVL rats,[134] and COX inhibition did not modulate vascular hyporesponsiveness of mesenteric vasculature in portal-hypertensive rats.[67] Therefore, the exact role of vasodilative prostanoids in the development and maintenance of splanchnic vasodilation in portal hypertension remains to be unraveled.

Finally, several studies indicate up-regulation of heme-oxygenases (HO) and associated increases in *carbon-monoxide (CO)* synthesis, which, contribute to the development and severity of arterial vasodilation in CBDL,[135] CCl_4,[136,137] and PVL rats.[138] Two isoforms of HO have been identified, of which HO-1 is inducible by multiple agents and HO-2 is expressed constitutively.[139] CO-induced vasodilation can occur through activation of soluble guanylate cyclase and cGMP production and also via Ca^{2+}-activated potassium channels. Increased expression of HO-1 but not HO-2 has been reported in the aortae and mesenteric arteries of CBDL rats.[135] Moreover, the selective inhibition of HO-activity, titrated to normalize aortic HO activity, has been shown to ameliorate the HCS in portal hypertension.[135] CO also seems to be involved in the pathogenesis of the HPS in CBDL rats because enhanced HO-1 expression in pulmonary monocytes is present and HO inhibition partially corrected the HPS in these animals.[140]

Impaired Contractile Signaling in VSMs

The previously stated development of the HCS in eNOS-knockout mice after the induction of experimental portal hypertension[29,63] evidences the presence of NO-independent vasodilation participating in the pathogenesis of this syndrome. Moreover, several perfusion studies in isolated vessels from various animal models of portal hypertension suggest a component of vascular hyporesponsiveness to vasoconstrictors, which is resistant to endothelial denudation.[89,141,142] Because the affinity and number of receptors for endogenous vasoconstrictors are at least not reduced during portal hypertension,[143–145] impaired contractile signaling at the subreceptor level has to account for the observed endothelium-independent and vasodilator-independent component of vascular hyporeactivity in portal hypertension.

Vasoconstrictor-induced Smooth Muscle Signaling Pathways

The contractile state of VSM cells depends essentially on the phosphorylation state of myosin light chains (MLCs). Contractile agonists usually increase MLC phosphorylation via the activation of MLC kinase or the inhibition of MLCP. Phosphorylated MLC in turn stimulates actin-myosin ATPase, resulting in the cross-bridging of actin and myosin that leads to smooth muscle contraction.[146,147] In contrast, vasodilative agents decrease MLC phosphorylation via indirect deactivation of MLC kinase or activation of MLCP. Under normal conditions, not only endogenous vasoconstrictors such as catecholamines and angiotensin but also vasopressin, endothelin, and leukotrienes act via stimulation of G-protein-coupled receptors. These receptors subsequently activate downstream effectors such as phospholipase C (PLC) and GTPase RhoA, leading to increased MLC phosphorylation. Activated PLC leads to the release of second messengers inositol-triphosphate (IP3) and diacylglycerol (DAG).[148] IP3 induces release of Ca^{2+} from the endoplasmic reticulum, and DAG targets protein kinase C (PKC), which modulates a wide assortment of cellular processes linked to the regulation of vascular tone.[149] As for activated RhoA, subsequent activation of RhoA kinase occurs, which by inhibition of

MLCP increases MLC phosphorylation and hence, the vascular contractile state (Fig. 6).

With respect to alterations in PLC-signaling, in fact impaired vascular reactivity to angiotensin in BDL rats, for example, includes reduced activation of PLC and subsequently diminished formation of IP3.[141] This reduction of PLC activity and, consequently, impaired DAG formation might also explain the findings of diminished activation of DAG-dependent PKC isoforms, which have been reported in aortae from CBDL rats.[150,151] Recent elegant studies have also unraveled alterations of RhoA signaling and its role in arterial vasodilation during portal hypertension. In CBDL rats, diminished Rho-kinase activity has been reported to impair vascular contractility in extrahepatic vessels and thus to contribute to splanchnic and systemic vasodilation.[145,152] This reduction in basal Rho-kinase activity seems to be due to posttranscriptional Rho-kinase down-regulation and impaired agonist-induced activation of residual Rho kinase. Consequences of reduced vascular Rho-kinase activity include a corresponding decrease in the phosphorylation of Ca^{2+}-sensitizing proteins, increase in MLCP activity, and reduced Ca^{2+} sensitivity, all of which lower the contractile state of VSM cells. In fact, the vasodilative effect of the Rho-kinase inhibitor Y-27632 in vivo (drop in systemic vascular resistance) and in vitro (vasorelaxation of adrenergic preconstricted aortae) has been shown to be greater in cirrhotic rats compared with control animals.[152] Whether these alterations in the intracellular signaling of VSM cells are similarly present and similarly expeditious in other models of portal hypertension, particularly in mesenteric arteries, remains to be seen. Finally, the question of whether these changes are the primary and, thus, causative factors initiating vascular dysfunction or whether they occur as a late phenomenon has to be answered by future studies.

Cotransmission of Vasoconstrictive Mediators

In most studies, vasoconstrictors are studied individually, focusing on the specific intracellular pathways and alterations seen in portal hypertension. However, in vivo, no vasoconstrictor acts alone. In general, the net vasoconstrictive effect is determined by the sum of a multitude of vasoconstrictors acting in concert to regulate vascular tone. In the following section, the potential modulation of each vasoconstrictor and its vasoconstrictive capacity by other vasoconstrictive agents will be discussed using the example of sympathetic vasoconstriction and the well known cotransmission of norepinephrine and neuropeptide Y (NPY).[153,154] This example is chosen because, in the mesenteric circulation in humans and in rodents, SNS-induced vasoconstriction is largely mediated by the activation of postsynaptic α_1 adrenoreceptors.[148,155] In fact, α_1-adrenergic stimulation represents the primary mechanism by which the SNS controls splanchnic vascular resistance.[148] In portal hypertension, some investigators even suggest that the most important functional vasoconstrictor defect is a loss

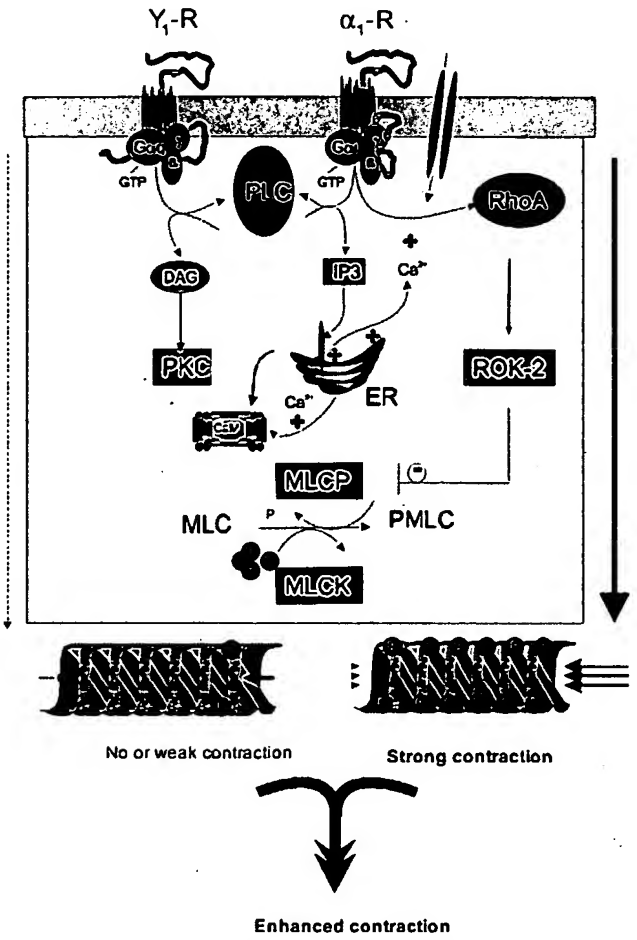

FIGURE 6. Vasoconstrictor-induced VSM signaling and cotransmission of α_1 adrenoreceptor and neuropeptide Y_1 receptor. Receptor activation leads to dissociation of the Gα protein from the receptor and its β/γ subunit activating the downstream effectors. These effectors, that is, PLC and the small monomeric GTPase, RhoA, are part of 2 different intracellular contractile signaling cascades. Both lead to MLC phosphorylation, however, via different mechanisms. PLC activation leads to the formation of IP3 and DAG. IP3 initiates VSM contraction by the opening of the IP3-regulated sarcoplasmatic Ca^{2+} channels. In parallel, DAG activates several isoforms of PKC, some of which are capable of contributing to vasoconstriction Ca^{2+} independently. Moreover, PKC increases the Ca^{2+} sensitivity of the VSM by inhibition of MLCP. Activated RhoA consecutively activates Rho kinase (ROK-2), which mediates the inhibition of MLCP. α_1-adrenergic agonists cause a rapid and potent PLC and RhoA stimulation and, thus, unequivocally in almost any vascular bed and species, cause a marked vasoconstriction. In contrast, Y_1-receptor activation per se only causes a slow and modest (independent of species and vascular bed) PLC activation. In the presence of α_1-adrenergic stimulation, however, the activation of Y_1 receptors leads to a potent amplification of vasoconstriction and, thus, cotransmission. The exact mechanisms responsible for this are not yet clear.

in adrenergic vascular tone within the intestinal microcirculation.[156]

NPY is costored with norepinephrine in presynaptic vesicles; however, in contrast to norepinephrine, its release necessitates a strong and sustained sympathetic nerval stimulation.[157] Biologic effects of NPY are mediated by different NPY receptors (Y1-Y6), of which mainly the Y1 receptors on the VSM are responsible for its vascular effects.[158] Y1 receptors represent G_i-protein-coupled receptors whose stimulation has been shown to potentiate norepinephrine-evoked vasoconstriction.[154,159–161] Such synergy is important in the regulation of vascular tone because it results in the sensitization of the direct-acting agonist, namely, in a leftward shift of the dose-response curve, or even in an increase in the maximal response.[162,163] For instance, blood vessels are sensitized to norepinephrine by pretreatment with NPY or by costimulation with concentrations of NPY, which on their own do not elicit potent contractile effects.[159,161,164–169] Moreover, NPY also facilitates other vasoconstrictive agonists such as angiotensin or vasopressin and, hence, causes profound pressor effects in vivo.[170,171] It has recently been evidenced in prehepatic experimental portal hypertension that NPY endothelium independently potentiates adrenergic vasoconstriction in mesenteric arteries via Y1 receptors, which is more pronounced than in sham animals.[172] In fact, in the presence of NPY, splanchnic vascular reactivity to α_1-adrenergic vasoconstriction was normalized in portal-hypertensive animals. This might indicate a profound lack of cross talk among sympathetic vasoconstrictors in the splanchnic circulation during portal hypertension.

Similar to NPY, the somatostatin analog octreotide (used widely for the treatment of acute variceal hemorrhage in cirrhotic patients) per se exerts no direct vasoconstrictive effect in mesenteric arteries. However, in the presence of vasoconstrictors that activate, PKC octreotide facilitates vasoconstriction and increases vessel resistance.[173] This type of cotransmission has been shown to be mediated by PKC-dependent activation of phospholipase A2 and the associated COX-derived production of vasoconstrictive prostanoids. Indeed, this mode of action explains the nearly immediate effect observed after bolus injections of somatostatin. Moreover, such cotransmission, requiring the presence of PKC-dependent vasoconstrictors, represents also the cellular basis for the observed local vasoconstrictive effect of octreotide on the forearm arterial vasculature in vivo in cirrhotic patients.[174]

SUMMARY

The basis for any experimental investigation delineating the mechanisms of arterial vasodilation in portal hypertension is the use of the most suitable animal model and experimental armamentarium applying to the most appropriate vessel/vascular bed. Only by considering the limitations and drawbacks of each method and of each animal model applied can straightforward results be obtained, which sustain further translational studies in patients and, thus, improve the treatment of portal hypertension. By doing so, Groszmann et al have evidenced several key aspects of arterial vasodilation in vitro and in vivo, including overproduction of humoral and endothelial vasodilators in the splanchnic circulation. In detail, eNOS-derived and nNOS-derived mesenteric NO overproduction has been evidenced in experimental portal hypertension. Moreover, eNOS up-regulation precedes the development of the HCS and occurs earliest at the intestinal mucosal microvasculature. These changes are most likely triggered at least in part by the up-regulation of VEGF expression and cyclic strain; however, other mechanisms might also be involved. In advanced stages of portal hypertension and liver cirrhosis, BT might further aggravate arterial vasodilation via additional augmentation of eNOS-derived NO synthesis. This effect seems to be mediated via the stimulation of GTPCH-I activity and, thus, BH4 synthesis that facilitates eNOS activity. However, other vasodilators such as EDHF, prostaglandins, CO, anandamide, etc. similarly contribute to the severity of splanchnic and systemic vasodilation in portal hypertension. Finally, recent investigations revealed defects in the contractile pathways of VSM cells, which contribute to vascular hyporesponsiveness to endogenous vasoconstrictors. Whether these latter changes in the contractile apparatus are late events in the development of the HCS, or are also causative early alterations that trigger the cascade of events leading to the HCS, and/or are influenced by vascular NO overproduction remains to be clarified.

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Splanchnic and Systemic Vasodilatation

The Patient

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Abstract: Arterial vasodilatation is one of the most important characteristics of cirrrhosis and portal hyptertension. Nowadays, it has been known that progressive vasodilatation is an essential factor contributing to hyperdynamic circulation and multiple organ dysfunction in liver cirrhosis. Over the past decades, numerous investigations have originated from the clinical observations. Clinicians and investigators have learned and applied new concepts of the pathophysiology of portal hypertension. For example, we now have effective pharmacologic treatment for hepatorenal syndrome. This review summarizes the developement of progressive vasodilatation syndrome in liver cirrhosis and portal hypertension with focus on the patients.

Key Words: vasodilatation, liver cirrhosis, portal hypertension

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Progressive vasodilatation is one of the characteristics observed in cirrhotic patients. With the progression of liver disease, an accentuation of this hemodynamic abnormality is a common phenomenon. In the recent decades, progressive vasodilatation in liver cirrhosis has been the focus of intensive investigation. There are several reasons for the research interest in this area. The first is its pathogenetic role in hyperdynamic circulation and portal hypertension. The second one is that this important syndrome leads to the development of the most common and potentially lethal complications of liver cirrhosis, namely gastroesophageal varices, spontaneous bacterial peritonitis, and portosystemic encephalopathy. Moreover, the mechanisms of action of the pharmacologic agents clinically available for the treatment of portal hypertension involve modulation of these hemodynamic aberrations.

Advanced liver cirrhosis is often accompanied by a hyperdynamic circulation. The clinical manifestations of hyperdynamic circulations are warm skin, capillary pulsations of the digits, cutaneous spider angioma, increased heart rate, reduced arterial pressure, wide pulse pressure, and bounding pulses. On the basis of these clinical observations, Kowalski and Abelmann[1] first systemically investigated the cardiovascular aberrations in alcoholic liver cirrhosis. They demonstrated that liver cirrhosis is associated with a hyperdynamic circulation, namely an increase in cardiac output and a decrease in peripheral vascular resistance. After the exclusion of various contributing factors, they concluded that the increased blood flow is due to peripheral vascular dilatation resulting from vasodilators produced by diseased livers. Over the next years, these observations were confirmed in the subsequent studies.[2–5] The presence of hyperdynamic circulation is related to liver failure[2] and can be observed in various types of portal hypertension.[2,6–10] Nowadays, progressive vasodilatation and hyperdynamic circulation have been recognized as important contributory factors to multiple organ dysfunction in patients with liver cirrhosis.[11] In fact, the hemodynamic evaluations of the magnitude of vasodilatation have been shown to be good prognostic indicators for cirrhotic patients in different clinical settings.[12–14] Although the overall systemic vascular resistance is decreased in cirrhotic patients, the individual vascular beds could be hyper-perfused, hypo-perfused, or normally perfused. These harmful effects of hemodynamic impairments on different vital organs are mediated by vasodilatations itself (such as in splanchnic and pulmonary circulation) or the response to vasodilatation in other vascular beds (such as in hepatorenal syndrome or chronic hepatoencephalopathy).

THE HYPERDYNAMIC SPLANCHNIC CIRCULATION

The splanchnic circulation includes the blood flow through the stomach, small intestine, large intestine, pancreas, spleen, and the liver. The splanchnic circulation is the main vascular bed responsible for the reduction in vascular resistance in portal hypertension state. Arterioles are the major sites of resistance to blood flow, thus regulating blood flow. Therefore, an increase in splanchnic blood flow in portal hypertension is the result of a marked vasodilatation of arterioles in splanchnic organs, which drain blood into the portal venous system. This striking hemodynamic aberration has been demonstrated

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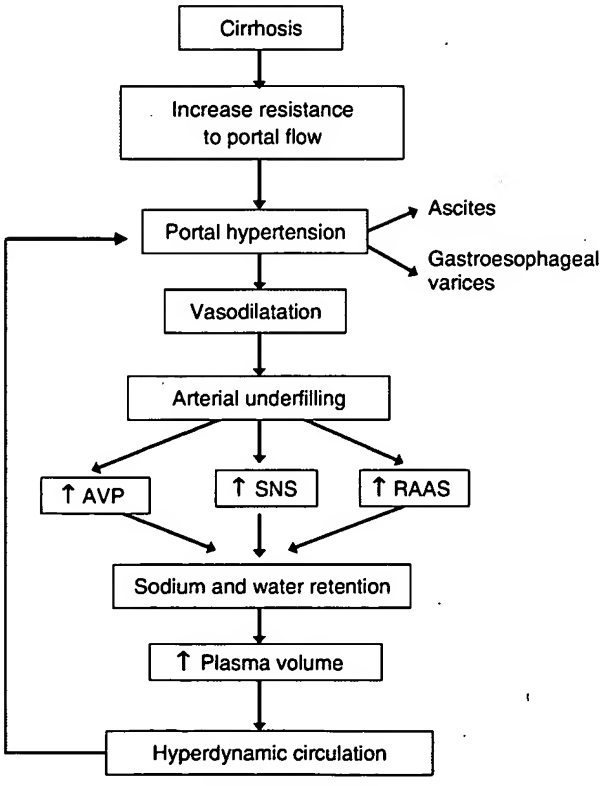
by the shortening of the mean transit time of labeled albumin in the splanchnic circulation of cirrhotic patients,[15] documentation of increased splenic and mesenteric blood flow in patients with portal hypertension.[6,16] Furthermore, the hyperdynamic splanchnic circulation, noninvasively assessed by a decrease of mesenteric artery impedance, occurs in cirrhosis since the early stage of liver disease and tends to deteriorate in relation to liver failure and the severity of portal hypertension.[17] From a hemodynamic point of view, the portal pressure is determined by the product of portal blood flow and vascular resistance to portal blood flow. In the normal subjects, portal blood flow through the liver accounts for nearly all the blood entering the splanchnic system. However, in portal hypertension state, perfusion of the liver by portal blood is decreased. In advanced stage of liver disease, splanchnic blood flow is diverted almost entirely through the collateral system, bypassing the liver and depriving it of portal blood flow. In the early 1980s, Groszmann et al[18] coined the name of "portal venous inflow" for the splanchnic blood flow entering the portal system to differentiate it from the portal flow perfusing the liver. Therefore, in portal hypertension, portal venous inflow consists of portohepatic blood flow plus the blood flow bypassing the liver through portosystemic collaterals.[19,20] Despite hepatic hypoperfusion with portal blood, the portal venous inflow is actually greatly increased in advanced stages of portal hypertension. Indeed, the increased portal venous inflow together with an increased resistance to portal inflow maintains and aggravates the portal hypertensive syndrome. This concept is called the "forward flow" theory. In contrast with the forward flow theory, the once dominant "backward flow" theory postulated that increased portal vascular resistance had paramount importance in elevating portal pressure, while blood flow was predicted to be hypodynamic and the portal system to be congested proximal to the obstruction. This concept was supported by studies reporting hypoperfusion of the liver in portal hypertension.[21,22] Nevertheless, the observation of a low portohepatic flow in advanced stage of portal hypertension can be deceptive because most of portal blood flow has been diverted to collateral circulation in that stage.

The azygos blood flow is especially important because the coronary-gastroesophageal collateral circulation drains into the azygos venous system in portal hypertensive patients.[23,24] It has been shown that azygos blood flow measured by a continuous thermal dilution technique can serve as an index of blood flow through gastroesophageal collaterals in liver cirrhosis.[25,26] Azygos blood flow has been shown to parallel portal hypertension and hyperdynamic circulation,[25,26] and an increased azygos blood flow is associated with an increase in the risk and severity of variceal bleeding.[26,27]

The splanchnic hyperdynamic circulation is mediated in part by vasodilatation, but arterial vasodilatation alone is not sufficient to cause the circulation to become hyperdynamic. Many vasodilators lower vascular resistance in normal humans or animals, but also cause relative underfilling of the circulation owing to increased vascular capacitance. In this situation, cardiac index either decreases or remains unchanged, rather than becoming hyperdynamic.[28–31] In conditions of constant peripheral vascular resistance, an increase in circulatory blood volume and thus mean circulatory filling pressure results in increased venous return and cardiac output.[32] However, acute expansion of blood volume leads to stress relaxation of the vasculature and cardiac output after initial increase returns to normal. This demonstrates that blood volume expansion alone is not sufficient in itself to maintain a hyperdynamic circulatory state. Taken together, it is the combination of arterial vasodilatation and blood volume expansion that produces optimal conditions for maintaining the hyperdynamic circulatory state in portal hypertension.

The peripheral arterial vasodilatation hypothesis offers a unifying explanation of the salient clinical features of the portal hypertensive syndrome[33] (Fig. 1). In brief, peripheral vasodilation leads to a decrease in central blood volume. This relative underfilling of arterial circulation is the physiologic stimulus for baroreceptor, activating the sympathetic nervous system, renin-angiotensin system, and release of antidiuretic hormone.



AVP: vasopressin
SNS: Sympathetic nervous system
RAAS: Renin-angiotensin-aldosterone system

FIGURE 1. The peripheral arterial vasodilatation hypothesis in liver cirrhosis and portal hypertension.

Mediators from these systems then result in sodium and water retention by the kidneys. Sodium retension is mainly due to increased tubular reabsorption of sodium mediated by receptors for angiotensin, aldosterone, and α-adrenergic stimuli,[34] whereas the impairment in water excretion is more complex and involves increased secretion of antidiuretic hormone, reduced delivery of filtrate to the ascending limb of the loop of Henle, and reduced renal synthesis of prostaglandins.[34]

Some maneuvers like transjugular intrahepatic portosystemic shunt, which can enhance central blood volume, are often associated with natriuresis and a further decline in systemic vascular resistance.[35] These observations have been sometimes used as evidence against primary arterial vasodilatation theory of sodium and water retention in liver cirrhosis. However, it is noteworthy to point out that a compensatory arterial vasodilatation occurs to buffer against the potential hypertensive effect that may be brought about by an increase in cardiac preload. This point can be clearly seen in portocaval anastomosis in experimental setting.[36] If the decreased systemic vascular resistance in portal hypertension was secondary to a primary volume expansion leading to an increase in cardiac output, there should be a tendency for the neurohumoral axis to be suppressed.

SYSTEMIC CIRCULATION AND THE HEART

Although the splanchnic circulation is the major vascular bed responsible for the reduction in vascular resistance, the systemic hemodynamics seems to be secondary to the alteration in splanchnic circulation. As a rule in cardiovascular physiology, the cardiac output levels vary reciprocally with the changes in total peripheral resistance.[37] One can easily understand this phenomenon by considering the Ohm's law (cardiac output = arterial pressure/total peripheral resistance). In fact, in human beings, the pathologically high cardiac output is almost always caused by chronically reduced total peripheral resistance.[37] Even though splanchnic arterial vasodilatation is consistently reported,[38,39] the studies concerning the extrasplanchnic arterial hemodynamics are conflicting. Palmar erythema, warm extremities, spider nevi, capillary pulsations, and high peripheral venous oxygen tension are common findings in cirrhotic patients. It suggests that the peripheral vasodilatation occurs in muscular and cutaneous vascular beds. Indeed, some plethysmography studies have shown that forearm blood flow in increased in cirrhotic patients.[7,40,41] However, evaluation of limb blood flow using color Doppler and spectral Doppler failed to demonstrate an obvious hyperdynamic circulation in this vascular territory.[42,43] The reasons for the discrepancy may be due to the different methods used to measure blood flow. Venous occlusion plethysmography of the limbs may give a combination measurement of cutaneous and muscular blood flow. Nowadays, it can be concluded that the cardiac output is increased in liver cirrhosis and portal hypertension. However, the precise allocation of the increased cardiac output to different organs may vary considerably. It is likely that the perfusion pattern of individual organ changes as the disease stage evolve through compensated liver cirrhosis, decompensated liver cirrhosis to a fully developed hepatorenal syndrome.[44,45] Although the splanchnic vasodilatation is consistent and parallel the severity of liver failure, the vascular tone of extrasplanchnic organs is subject to the activity of neurohumoral system that is activated by splanchic vasodilatation. In this context, the cardiac dysfunction may play an important role in the progression of this vasodilatation syndrome and is worthy of mention.

The term of cirrhotic cardiomyopathy has been coined to describe the impaired cardiac function associated with liver cirrhosis.[46] The hallmark of the cardiac dysfunction in liver cirrhosis is a baseline hyperdynamic systolic function with a blunted cardiac response to various stress situations.[46–49] The hyperdynamic systolic function is characterized by an enhanced baseline left ventricle ejection fraction and stroke volume index. This hyperdynamic systolic function along with activated neurohumoral system acts as a compensatory mechanism to maintain the arterial pressure in the face of progressive vasodilatation in portal hypertension. Basal diastolic dysfunction and left ventricle hypertrophy have also been reported in several studies.[50,51] It is speculated that continued hemodynamic overloading, neuroendocrine activation, and a diminished myocardial β-adrenergic receptor signal transduction function are responsible for these functional and structural changes.[46] The latent cardiac dysfunction may become unmasked as the progressive vasodilatation overwhelms the compensatory mechanisms. After the insufficient cardiac output becomes evident, arterial pressure homeostasis can depend only on the activity of neuroendocrine system, which may exert harmful effects on extrasplanchnic circulation such as renal vascular bed. The clinical significance of such cardiac dysfunction is also documented in other disease entity associated with hyperdynamic circulation. For example, it has been shown that inadequate increase in cardiac output is associated with an increased mortality rate in patients with severe sepsis.[52] Interestingly, the cardiac dysfunction along with the systemic hyperdynamic state can be reversed by liver transplantation.[50,51,53]

THE HYPERDYNAMIC PULMONARY CIRCULATION

The vasodilatation syndrome also has impacts on the lungs. Investigations have shown that as many as 40% of cirrhotic patients have evident intrapulmonary vasodilatation.[54] Intrapulmonary vasodilatation is associated with hepatopulmonary syndrome, one of the most important complications of liver cirrhosis.[55] Hepatopulmonary syndrome is characterized by an impaired arterial oxygenation and can influence the prognosis of cirrhotic patients.[55,56] Although a correlation between the severity of esophageal varices and the hepatopulmonary

syndrome has been suggested,[57] subsequent study cannot demonstrate a clear relation among the severity of liver disease, clinical features, or arterial hypoxemia in patients with the hepatopulmonary syndrome.[58,59] Recently, Katsuta et al[60] showed an association between hyperdynamic pulmonary blood flow and impaired arterial oxygenation in patients with chronic liver disease, suggesting the pathogenetic role of shear stress in hepatopulmonary syndrome. However, this aspect needs further investigation. Impaired arterial oxygenation in hepatopulmonary syndrome is caused by ventilation perfusion mismatching and intrapulmonary shunt along with diffusion-perfusion impairment. Diffusion-perfusion impairment is a proposed mechanism of hypoxemia associated with intrapulmonary vascular dilatations. In brief, because the capillary is dilated and has an expanded diameter, oxygen molecules from adjacent alveoli cannot diffuse to the center of the dilated vessel to oxygenate hemoglobin in erythrocytes at the center stream of venous blood.[61] Some investigations using inert gas elimination technique support the presence of diffusion impairment in cirrhotic patients with hepatopulmonary syndrome.[62,63] At present, overproduction of vasodilators in pulmonary vasculature is believed to play an important role in the pathogenesis of pulmonary vasodilatation and hepatopulmonary syndrome.[55,64,65] However, additional mechanisms such as neovascularization and remodeling have been suggested.[66]

THE RENAL CIRCULATION

A normal renal perfusion flow is essential to maintain an adequate glomerular filtration rate and excretion functions. Under physiologic situation, the renal perfusion is maintained within normal limits through sophisticated mechanisms, involving a balance between local vasodilators and vasoconstrictors.[67] This balance in renal circulation is especially important in the setting of liver cirrhosis. Under some circumstances, the balance cannot be maintained, hence making vasoconstrictors predominate over the vasodilators. Early investigations have shown that the renal blood flow is diminished in patients with advanced liver cirrhosis.[68,69] Subsequent studies showed that patients with less advanced cirrhosis may have renal vasoconstriction of less magnitude that tends to progress over time.[70,71] Hepatorenal syndrome can be considered as the extreme expression of renal vasoconstriction.[70,71] The exact mechanisms behind renal vasoconstriction in liver cirrhosis are not fully understood. The renal hypoperfusion in liver cirrhosis seems to be the consequence of hyperdynamic circulation. In this context, the peripheral arterial vasodilatation theory mentioned earlier can offer an explanation for renal vasoconstriction in advanced liver cirrhosis. In brief, at advanced stage of liver disease, the renin-angiotensin-aldosterone, sympathetic nervous systems, and antidiuretic hormone have been strikingly activated. As the splanchnic vasculature is resistant to angiotensin II, norepinephrine, and vasopressin due to locally produced vasodilators,[72] the maintenance of arterial pressure can only be achieved by vasoconstriction in extrasplanchnic vasculatures such as renal circulation. This compensatory vasoconstriction induced by neurohumoral factors may become even more pronounced and finally detrimental when another compensatory factor, increased cardiac output, begins to fail.

Recently, 2 studies by Ruiz-del-Arbol et al[73,74] lent support to this point. Recent investigations have demonstrated that concomitant administration of albumin and preferential splanchnic vasoconstrictors such as terlipressin improves renal function in patients with hepatorenal syndrome.[75,76] These observations can provide indirect evidence for peripheral arterial vasodilatation theory.

THE CEREBRAL CIRCULATION

The role of cerebral blood flow in the brain dysfunction of liver failure has not been well established. However, cerebral hypoperfusion may impair the brain function if oxygen delivery becomes inadequate, whereas cerebral hyperperfusion may induce brain edema. Under physiologic conditions, the cerebral blood flow is efficiently autoregulated.[77] Cerebral autoregulation ensures a constant brain blood flow regardless of wide changes in arterial pressure.[77] The cerebral perfusion is also regulated by brain metabolism.[78] In patients with acute liver failure, the cerebral autoregulation is no longer present.[79] Therefore, the cerebral perfusion changes along with the alteration of arterial blood pressure. Both increase and decrease in cerebral blood flow have been reported in acute liver failure.[80] The cerebral autoregulation is often impaired in liver cirrhosis with ascites as well.[81] In fact, it has been demonstrated that the resistant index of cerebral artery is increased in cirrhotic patients with ascites, indicating a cerebral vasoconstriction.[82] The degree of cerebral vasoconstriction was shown to correlate with that of renal vasoconstriction and plasma renin activity, suggesting the mechanisms of vasoconstriction in these two end organs may be similar. It is unknown whether the effective treatment for hepatorenal syndrome has beneficial effects on cerebral vasoconstriction in liver cirrhosis.

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Portal Hypertension and Its Complications




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Portal hypertension is a clinical syndrome defined by a portal venous pressure gradient exceeding 5 mm Hg.[1] Cirrhosis is the most common cause of portal hypertension in the Western world.[1] The goal of this review is to provide an overview of the current understanding of the pathophysiology and treatment of portal hypertension.

Pathogenesis of Portal Hypertension: Hemodynamic Factors

The hallmark of portal hypertension is a pathologic increase in the pressure gradient between the portal vein and the inferior vena cava, which is measured by the hepatic venous pressure gradient (HVPG).[2] Briefly, the wedged hepatic vein pressure (WHVP), a marker of sinusoidal pressure, and the free hepatic vein pressure (FHVP) are measured with radiologic assistance. HVPG is calculated by the following formula[2–4]:

$$\text{HVPG} = \text{WHVP} - \text{FHVP} \quad (1)$$

The FHVP is subtracted from the WHVP to correct for intra-abdominal pressure to provide an accurate measure of the portal vein pressure. As in any other vessel, the pressure within the portal vein is determined by the product of blood flow and resistance to its egress, as defined by Ohm's law (Figure 1):

$$P\,(\text{pressure}) = Q\,(\text{blood flow}) \times R\,(\text{resistance}) \quad (2)$$

Portal hypertension is initiated by increased outflow resistance; this can occur at a presinusoidal (intra- or extrahepatic), sinusoidal, or postsinusoidal level. As the condition progresses, there is a rise in portal blood flow, a combination that maintains and worsens the portal hypertension.[5]

Increased Hepatic Vascular Resistance: Structural and Dynamic Components

In cirrhosis, the principal site of increased resistance to outflow of portal venous blood is within the liver itself. This results from 2 factors: (1) mechanical obstruction to flow because of fibrotic disruption of architecture and (2) a dynamic component produced by active contraction of vascular smooth muscle cells and activated stellate cells.[1,6–8] Although the former is not acutely modifiable, disease stabilization and improvement, eg, after successful treatment of hepatitis C or abstinence from alcohol, can improve fibrosis and the mechanical component.[9] The dynamic component accounts for approximately 30% of the intrahepatic resistance in cirrhosis and is an important target for future therapy.[10]

Mechanism of Increased Hepatic Vascular Tone: Intrahepatic Endothelial Dysfunction

Cirrhosis is associated with evidence of endothelial dysfunction, both in the systemic circulation and within the liver.[11,12] The net effect in the liver is intrahepatic vasoconstriction. This is mediated by decreased endothelial nitric oxide synthetase (eNOS) activity and NO production.[12–14] Hepatic eNOS activity is decreased because of impaired Akt-mediated eNOS phosphorylation (which is partially reversible by statins) and increased caveolin expression (particularly if folate deficiency exists).[15–17] Other factors that contribute to intrahepatic vasoconstriction include decreased NO

Abbreviations used in this paper: ADH, antidiuretic hormone; EVL, endoscopic variceal ligation; GOV, gastroesophageal varices; HE, hepatic encephalopathy; HRS, hepatorenal syndrome; HVPG, hepatic venous pressure gradient; LVP, large volume paracentesis; SBP, spontaneous bacterial peritonitis; TIPS, transjugular intrahepatic portasystemic shunts; VEGF, vascular endothelial growth factor.

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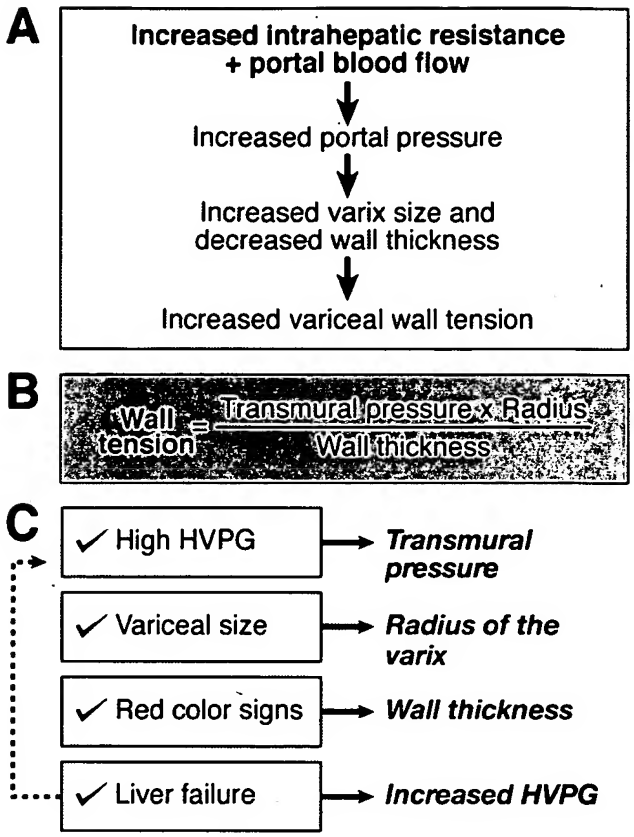

Figure 1. Pathophysiology of variceal bleeding. Bleeding occurs when the tension exerted by the thin wall of the varices exceeds the rupture point. This is facilitated by the progressive increase in the size of the varices and decreased wall thickness (*A*). These factors are mathematically interrelated in Laplace's law (*B*) and explain why an increased HVPG, the endoscopic apearance of the varices, and the degree of liver failure are associated with increased risk of variceal bleeding (*C*).

availability because of its utilization for nitrosylation reactions secondary to oxidative stress[18] and vasoconstriction mediated by endothelin, angiotensinogen, and eicosanoids.[18,19] The role of several other vasoactive mediators such as carbon monoxide, adrenergic tone, endotoxemia, and inflammatory cytokines are currently under investigation.

Increased Portal Venous Inflow

Mesenteric arterial vasodilation is a hallmark of cirrhosis and contributes to both increased portal venous inflow and a systemic hyperdynamic circulatory state (low systemic vascular resistance and mean arterial pressure with high cardiac output).[5,20] Increased NO production because of increased eNOS activity in the systemic circulation is a major driver of arterial vasodilation.[21] Shear stress, increased vascular endothelial growth factor (VEGF), and tumor necrosis factor-α are causes of increased splanchnic NO production in cirrhosis.[22–24] Increased heme oxygenase activity and CO production may also contribute to the hemodynamic disturbances.[25] Bacteremia can increase vasodilation by stimulating tumor necrosis factor-α production and activation of endocannabinoids, which are potent vasodilators.[26] Blockade of VEGF signaling attenuates the increase in portal venous inflow seen in cirrhosis.[27]

Formation of Varices and Mechanism of Variceal Hemorrhage

Nature decompresses the hypertensive portal vein by diverting up to 90% of the portal flow through portasystemic collaterals back to the heart, resulting in flow-mediated remodeling and enlargement of these vessels. VEGF, NO-driven VEGF type II receptor expression, and platelet-derived growth factor drive this process.[21,28] A common location for such vessels is at the gastroesophageal junction at which they lie immediately subjacent to the mucosa and present as gastric and esophageal varices. Varices do not form until the HVPG exceeds 10 mm Hg and usually do not bleed unless the HVPG exceeds 12 mm Hg.[29,30]

Variceal rupture occurs when the wall tension exceeds the elastic limits of the variceal wall (Figure 1). The wall tension is defined by Frank's modification of Laplace's law[31]:

$$T = (P_{\text{varices}} - P_{\text{esophageal lumen}}) \times (\text{radius of varix})/\text{wall thickness} \quad (3)$$

The variceal pressure is dependent on variceal flow and resistance to outflow (see equation 2 above). Variceal flow is driven by the severity of portal hypertension. Thus, a high portal pressure and the variceal diameter are major determinants of variceal hemorrhage; an HVPG > 20 mm Hg has been associated with continued bleeding and failure of medical therapy in acute variceal hemorrhage.[32] In addition, varices are most superficial at the gastroesophageal junction and thus have the thinnest wall in that region: consequently, esophageal variceal hemorrhage invariably occurs in this region.[33]

Development of Ascites

Ascites is a common complication of cirrhosis.[34] Increased hepatic sinusoidal pressure is an essential prerequisite for the development of ascites. Three interrelated pathophysiologic processes contribute to the development of ascites. These include systemic arteriolar vasodilation, activation of Na and H_2O retention, and sinusoidal portal hypertension.

Systemic Arteriolar Dilation: Its Consequences and Role in Development of Ascites

Cirrhosis is associated with systemic arteriolar dilatation[35] (Figure 2). Systemic arteriolar dilatation increases the fraction of the total capillary bed in the body open for perfusion resulting in decreased filling of the

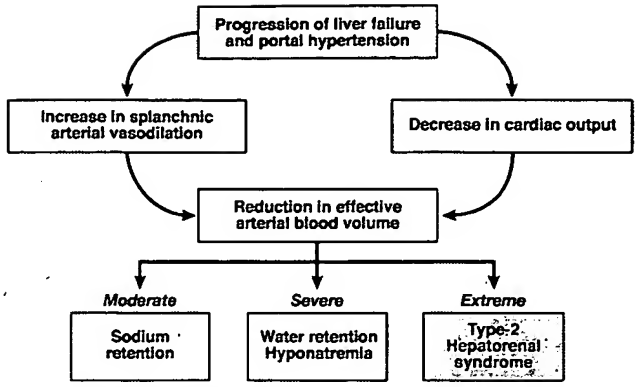

Figure 2. Pathophysiology of ascites and hepatorenal syndrome. The initial event is splanchnic arterial vasodilation, which causes effective hypovolemia. This is compensated for by an increased cardiac output (hyperdynamic circulation). However, as the disease progresses, splanchnic arterial vasodilation increases and cardiac output decreases, leading to deterioration of circulatory function and stimulation of the renin-angiotensin-aldosterone system, sympathetic nervous system, and antidiuretic hormone. When circulatory dysfunction is moderate, patients develop sodium retention. When it is severe, patients develop a profound impairment in free water excretion and dilutional hyponatremia. Finally, when it is extreme, patients present with HRS.

available vascular space. This is known as effective hypovolemia.

Another consequence of systemic arteriolar vasodilation is a decrease in mean arterial pressure. Increased heart rate and cardiac output tends to compensate for the decreased systemic vascular resistance and maintains mean arterial pressure, especially early in the course of the disease.[20] As arteriolar vasodilation worsens with disease progression, cardiac output fails to rise further.[36] In fact, the inotropic and chronotropic functions are impaired, and the cardiac output, although high in absolute terms, is disproportionately low for the degree of vasodilation.[36] This is accompanied by intense activation of endogenous vasoconstrictive mechanisms such as the renin-angiotensin, sympathetic nervous system, and the antidiuretic hormone (ADH).[37] Although unable to reverse the mesenteric arteriolar vasodilation, they produce severe vasoconstriction in other vascular beds, eg, the kidneys, brain, muscle, and skin.[38,39] The renal arteries are very sensitive to the vasoconstrictive effects of angiotensin II, norepinephrine, and ADH. This explains the low glomerular filtration rate and renal perfusion pressure with progression of cirrhosis; when the glomerular filtration rate is decreased enough to cause overt renal failure, hepatorenal syndrome (HRS) is considered to be present.[40]

Activation of Na and Water Retentive Mechanisms

Effective hypovolemia activates the renin-angiotensin-aldosterone pathway and sympathetic nerve activity. Both cause renal Na and water retention (Figure 2). These pathways are activated late in the course of ascites,[41,42] suggesting that there are yet other undiscovered mechanisms that are operative early in the course of cirrhosis. ADH secretion increases with more profound vasodilatation, resulting in water retention and hyponatremia.[43] Hyponatremia is a marker for advanced disease and is an independent predictor of outcome.[44]

Increased Sinusoidal Pressures: Local Mechanisms for Ascites Formation

Increased sinusoidal hydrostatic pressure leads to increased fluid movement from the sinusoids to the space of Disse, thereby increasing hepatic and thoracic duct lymph flow, which can be as much as 24-fold elevated vs normal.[45] Both increased outflow resistance and portal venous inflow contribute to sinusoidal hypertension and the formation of splanchnic lymph. When lymph production exceeds the capacity of the lymphatics to return it to circulation, the excess lymph spills into the peritoneal cavity. This is initially reabsorbed via microscopic stoma on the peritoneal surface of the diaphragm that communicates with supradiaphragmatic lymphatics.[46] When ascites formation exceeds its reabsorption, clinically evident ascites occurs.

Cirrhosis is also associated with a closing of the normal fenestrae and the deposition of a basement membrane below the sinusoidal endothelium.[47] This decreases sinusoidal endothelial permeability. Thus, for a given elevation of sinusoidal hydrostatic pressure, the ascites that is formed has a low protein and albumin concentration. A serum to ascites albumin gradient >1.1 suggests the presence of portal hypertension with cirrhosis.[48] Low levels of ascites proteins and opsonins increase the risk of spontaneous bacterial peritonitis (SBP).[49,50]

Table 1. Definition of Refractory Ascites and Hepatorenal Syndrome

Refractory ascites
Diuretic resistant ascites: Ascites that is difficult to mobilize, as defined by a failure to lose at least 1.5 kg/week of fluid weight, despite maximal diuretic therapy with spironolactone (400 mg/day) and furosemide (160 mg/day) or an equivalent dose of a distal-acting and loop-acting diuretic, respectively
Diuretic intractable ascites: Ascites that is difficult to mobilize, as defined above, because of the inability to provide effective doses of diuretics because of diuretic-induced adverse effects, eg, azotemia, hyponatremia, and others
Hepatorenal syndrome
Presence of cirrhosis with ascites
Presence of renal failure (creatinine level >1.5 mg/dL or 133 mol/L)
Lack of improvement in serum creatinine after 48 hours of diuretic withdrawal and volume expansion with intravenous albumin administration (1 g/kg/day up to 100 g/day)
Absence of shock
Use of nephrotoxic drugs, eg, aminoglycosides
Parenchymal renal disease (urine protein >500 mg/day, granular or red cell casts, hematuria, urinary obstruction by sonography)

NOTE. Based on International Ascites Club Criteria.[51,52]

Refractory Ascites and HRS

Initially, ascites is manageable with Na restriction and diuretic therapy. However, over time, some patients cease to respond even to maximal diuretic therapy and are considered to have refractory ascites.[51] This is associated with further exaggeration of the pathophysiologic mechanisms outlined above and decreased glomerular filtration rate, which may manifest as overt renal failure[52] (Table 1).

HRS is functional renal failure that occurs because of marked mesenteric arterial vasodilation, impaired cardiac response to the vasodilation, and increased renal vasoconstriction.[52] Increased angiotensin, sympathetic nerve activity, and ADH all contribute to renal vasoconstriction. Initially, these are compensated for by intrarenal vasodilatory mechanisms (prostaglandins, NO, and others).[40] As the renal balance between vasodilation and vasoconstriction tilts toward vasoconstriction, renal perfusion and glomerular filtration rate decrease. This process occurs in the transition from diuretic-responsive ascites to refractory ascites to HRS, and these states often form a clinical continuum rather than distinct clinical-pathophysiologic entities. The progression to and rate of development of renal failure is often accelerated by intermittent bouts of infection, particularly SBP, which are associated with increasing vasodilatation and impaired cardiac response to the vasodilatation.[53] These, in turn, further activate vasconstrictive pathways, causing renal vasoconstriction. Depending on the rate of development of renal failure, HRS is classified as type 1 (rapidly progressive) or type 2 (slowly progressive).[52] Type 2 HRS is usually seen in the context of refractory ascites. Type 1 HRS is associated with worsening hepatic function. The latter is believed to be due to decreased hepatic blood flow from increased sinusoidal resistance secondary to angiotensin-II, norepinephrine, and ADH-mediated stellate cell contraction.[36,53]

Hepatic Encephalopathy

Hepatic encephalopathy (HE) is a broad entity that encompasses mental status changes in subjects with acute and chronic liver failure. Variable degrees of hepatocellular failure and portal-systemic shunting are the anatomic substrate of HE, although either one can produce HE. Several mechanisms have been implicated in the genesis of HE and are reviewed below.

Interorgan Ammonia Metabolism

Ammonia is a key factor in the pathogenesis of HE.[54] In cirrhosis, decreased hepatic uptake of ammonia occurs as a result of intrahepatic portal-systemic shunts and/or reduced urea and glutamine synthesis. A substantial portion of gut-derived ammonia originates in the small bowel from the deamination of glutamine by glutaminase, which is activated in cirrhosis.[55] The potential importance of this enzyme is exemplified by its sensitivity to neomycin, which is used to treat HE.[56]

An often ignored regulator of circulating ammonia levels is the muscle mass. Striated muscle form glutamine from ammonia, which is later circulated to other organs.[57] Decreased muscle mass is often present in cirrhosis; it is associated with decreased muscle capacity to clear ammonia and further contributes to hyperammonemia. Physical activity releases ammonia from muscle and may also contribute to hyperammonemia.[58] Ornithine-aspartate, used for the treatment of HE, increases muscle glutamine synthetase activity via transcriptional activation and improves the elimination of NH_3 in anhepatic animal models.[59]

Recent studies have focused on the potential role of the kidney in NH_3 homeostasis. Gastrointestinal bleeding and hypovolemia increase release of renal ammonia to the circulation, whereas volume loading and sinusoidal decompression decrease such release.[60,61] The normal regulation of urinary ammonia excretion is complex and includes roles for ammoniagenic enzymes as well as different transporters.[62] A novel role for Rh glycoproteins, RhBG and RhCG, includes ammonia transport in the kidney[63] as well as in other mammalian cells, including the liver.[64] Several aquaporins may also participate and/or facilitate ammonia transport into cells.[65]

Role of Infections and Systemic Inflammation

Infection, which promotes inflammation, can precipitate HE.[66,67] Inflammation-induced neurologic dysfunction may result from endothelial activation by infection-induced circulating cytokines, cerebral sequestration of macrophages, altered microglial function, and interactions between cytokines and ammonia.[68-70] The therapeutic benefits of nonabsorbable antibiotics given orally may include decreased bacterial translocation and activation of inflammatory mechanisms.[71]

Cerebral Blood Flow

Normally, the cerebral cortex receives the bulk of the cerebral blood flow. Positron emission tomography scans, using ^{15}O (flow) and ^{13}N (ammonia metabolism), show diversion of blood flow to basal areas along with increased ammonia metabolism and decreased glucose utilization.[72] An inverse relationship between systemic arterial vasodilation and cerebral blood flow has been identified as well. The pathogenesis of cerebral vasoconstriction may be similar to that for renal vasoconstriction in cirrhosis with portal hypertension.[73]

Brain Edema and the Spectrum of HE

Intracranial hypertension can occur in cirrhosis but is rare. However, an increase in brain water content and low-grade brain edema occurs commonly in cirrhosis.[74] These changes are reversed with lactulose and liver transplant.[74,75] It is believed that increased osmotically

active solutes in the brain, eg, Na, glutamine, and myo-inositol, may play a pathogenic role.[76] The activation of compensatory mechanisms that tightly regulate cerebral osmolyte levels may explain the lack of clinically obvious cerebral edema in cirrhosis.

Oxidative Stress and HE

In the brain, ammonia is detoxified in the cytoplasm of astrocytes to form glutamine. Glutamine is transported to mitochondria where glutaminase activity releases ammonia.[77] This generates reactive oxygen species, which can induce the mitochondrial permeability transition, thereby resulting in mitochondrial and glial dysfunction (Figure 3).[78,79] This pathway has also been described as the "Trojan horse" hypothesis for cerebral dysfunction in HE.[77] Increased heme oxygenase-1 activity may be important as a source of CO production and modulation of cerebral blood flow.[80]

Hepatic Parkinsonism

Over 20% of subjects awaiting liver transplantation exhibit features of Parkinsonism.[81] This is associated with increased manganese deposition in the globus pallidus,[82] which is known to induce oxidative stress by altering mitochondrial function.[83]

Management of Portal Hypertension

Variceal Hemorrhage

Management of the subject who has never bled from varices. *Assessment of bleeding risk and identification of those who need intervention.* The risk of bleeding from esophageal varices depends on the HVPG (>12 mm Hg), variceal diameter, endoscopic "red signs," and liver failure.[30,84] Subjects with medium to large varices as well as those with Child-Pugh class B or C cirrhosis and varices of any size are considered to be at high risk of bleeding.[85] Although liver function, platelet count, and splenomegaly are related to the risk of having such varices, they cannot be used to guide the need for endoscopy at this time.[86,87] The risk of de novo development of "high risk" varices is 1% at 1 year and 9% by 3 years.[88] All subjects with cirrhosis should undergo a screening endoscopy to determine their risk of bleeding (Figure 4).[89] Subjects with "high risk" varices should be targeted for primary prophylaxis. Those without varices should have follow-up endoscopy in 2 years or at the time of clinical decompensation. Those with small varices and preserved hepatic function (low-risk varices) should have repeat endoscopy at 1-year intervals.[89]

Primary prophylaxis of variceal hemorrhage. Nonselective β-blockers produce mesenteric arteriolar vasoconstriction and thus decrease portal pressure. They reduce the risk of bleeding from 25% to 15% (relative risk reduction, 40%; number needed to treat (NNT), 10).[90] The best predictor of success is a sustained decrease in HVPG to values less than 12 mm Hg; those with a sustained 20% decrease in HVPG but to values above 12 mm Hg have a risk of bleeding under 10%.[91,92] The use of β-blockers is limited by the small number of subjects who have a hemodynamic response (~20%-30%), intolerance to therapy (~10%-20%), and rebound portal hypertension if discontinued suddenly. Combination therapy with β-blockers and nitrates cannot be recommended because of the discrepant results of clinical trials. Endoscopic variceal ligation (EVL) reduces the risk of bleeding and

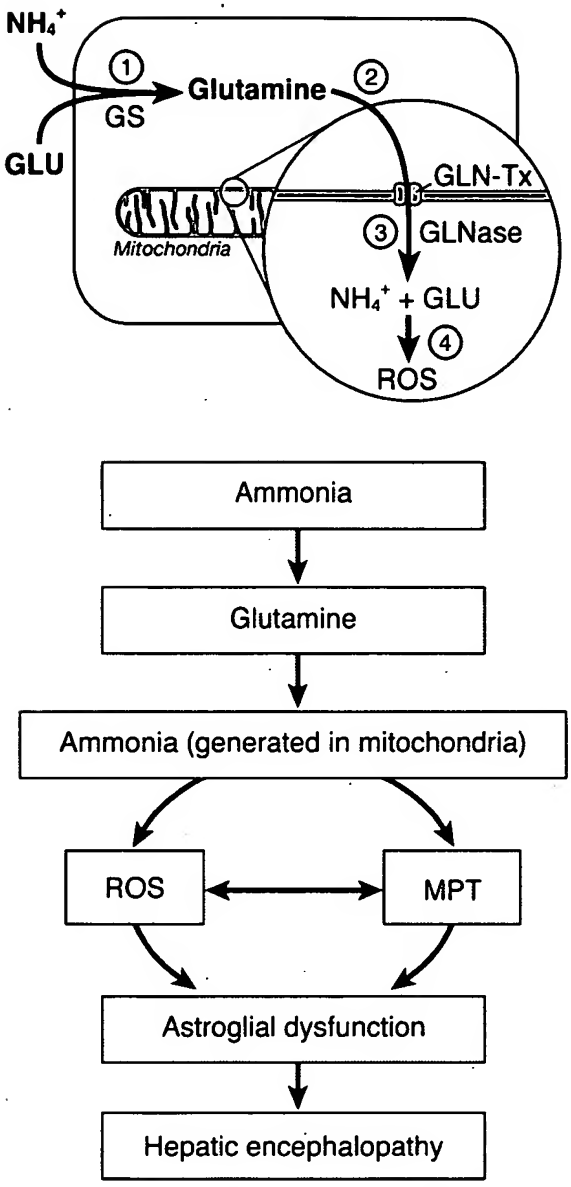

Figure 3. Glutamine as a "Trojan horse." In astrocytes, glutamine is transported into mitochondria via specific transporters, where under the action of glutaminase, ammonia is regenerated. Mitochondrial dysfunction occurs, with the generation of reactive oxygen species (ROS) and the mitochondrial permeability transition. Astroglial dysfunction leads to altered glial-neuronal communications, with abnormalities in neurotransmission (eg, glutamate, GABA) and the development of encephalopathy.

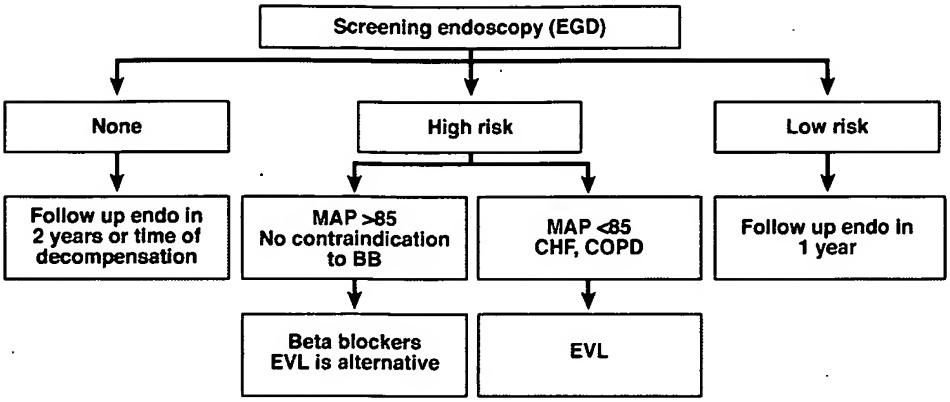

Figure 4. An algorithm for the primary prophylaxis of variceal hemorrhage.

improves survival compared with no treatment.[93] Meta-analysis of trials of EVL vs β-blockers show that EVL reduces the risk of bleeding from 23% to 14% with an NNT of 11.[94] However, the survival was similar to that with β-blockers. These 2 treatments are therefore comparable.

For patients with high-risk varices and no contraindications to the use of β-blockers, β-blockers are the usual first-line treatment of choice, although EVL represents an effective alternative (Figure 4). EVL is often used as the first-line treatment in those who have a contraindication for the use of β-blockers or risk factors for intolerance to β-blockers. There is increasing interest in an a la carte approach to primary prophylaxis, which is guided by the HVPG response to initial β-blocker treatment.[95] For those with a hemodynamic nonresponse (HVPG drop less than 20% and to values over 12 mm Hg), nitrates or EVL are added in this approach. The clinical utility and cost-effectiveness of this approach remains to be fully defined.

Management of active hemorrhage. The mortality from active hemorrhage has declined over the last decade to approximately 15%–20%.[34] Only 40%–50% of all active bleeds cease to bleed spontaneously. Any bleeding that occurs more than 48 hours after the initial admission for variceal hemorrhage and is separated by at least a 24-hour bleed-free period is considered to represent rebleeding.[96] Rebleeding that occurs within 6 weeks of onset of an acute bleed represents early rebleeding, and bleeding episodes that occur at later times are defined as late rebleeding episodes.

General measures. Packed red cells are transfused to keep the target hemoglobin after transfusion around 9 gm/dL (hematocrit: 25%–30%); overtransfusion increases the risk of rebleeding.[97] Fresh frozen plasma and platelets, although frequently used, do not reliably correct coagulopathy and can induce volume overload.[98,99] Recombinant factor VII has not been found to improve survival.[100] Airway protection should be provided as required. Empiric use of a third-generation cephalosporin, given intravenously, improves the outcomes of active variceal hemorrhage[101] (Table 2).

Control of bleeding. Although terlipressin, a synthetic analogue of vasopressin, and somatostatin are effective in controlling bleeding, they are not available in the United States.[102,103] A combination of endoscopic treatment (usually EVL) and pharmacologic treatment (octreotide in the United States) is the preferred first-line treatment to achieve hemostasis[89,104–106] (Figure 5).

Continued severe hemetemesis with or without hypotension and the need for continued transfusion to maintain the hematocrit are all markers of failure to control active bleeding.[96] The severity of portal hypertension (HVPG > 20 mm Hg), sepsis, and overtransfusion have all been linked to the risk of failure to control bleeding and early rebleeding.[32,107] The mortality associated with active variceal hemorrhage rises exponentially with continued bleeding.

EVL may be attempted once more for early rebleeding, but the decision to use this must be weighed against the risks of complications and the need to provide definitive

Table 2. General Measures for the Management of Active Variceal Hemorrhage

Airway protection
Endotracheal intubation if altered mental status or unconscious
Gastric aspiration
Hemodynamic resuscitation
Crystalloids and blood transfusion
Correction of coagulopathy and thrombocytopenia
Antibiotic prophylaxis for spontaneous bacterial peritonitis
Blood cultures and diagnostic paracentesis if ascites present
Third-generation cephalosporin intravenously and switch to oral quinolone when patients stable and GI tract is functional
Renal support
Urine output above 50 mL per hour
Avoid nephrotoxic drugs
Metabolic support
Injectable thiamine when indicated
Monitoring and treating delerium tremens
Monitoring and treating acid base and electrolyte disturbances
Monitoring blood glucose level
Neurologic support
Monitor mental state
Avoid sedation

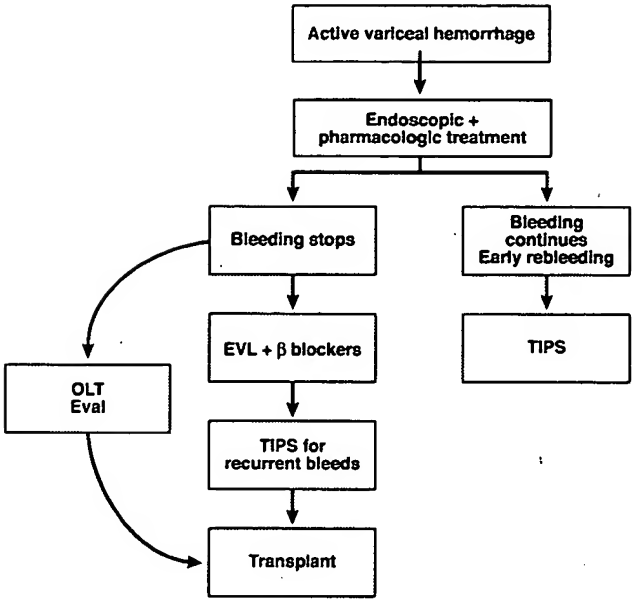

Figure 5. An algorithm for the management of variceal hemorrhage. Initial treatment is with endoscopic and pharmacologic treatment. Continued bleeding or severe early rebleeding can be managed with TIPS. Those who respond to first-line treatment should receive band ligation and β-blockers. A transplant evaluation should be initiated based on the standard of care locally. Varices should be ligated to obliteration, and suitable patients should undergo transplantation when an organ is available. TIPS is used as a salvage treatment for recurrent bleeding. Liver transplantation remains the only treatment that corrects both the portal hypertension and the underlying liver disease and is the definitive treatment of choice in the long-term for appropriate candidates.

therapy. Balloon tamponade can effectively produce temporary hemostasis in 80%–90% of cases.[108,109] Transjugular intrahepatic portasystemic shunts (TIPS), a radiologic procedure by which a tract is created between the hepatic and portal vein and kept open by deployment of a coated stent, is the salvage procedure of choice in most subjects.[110-112] TIPS produces hemostasis in over 90% of cases and is effective both for gastric and esophageal variceal bleeding.[112]

Prevention of recurrent bleeding. EVL reduces the relative risk (vs sclerotherapy) of rebleeding by 37% and the absolute risk by 13% (NNT, 8).[113] Nonselective β-blockers reduce the relative risk of bleeding by 33% with an NNT of 4.76.[90] Combination therapy of EVL and β-blockers is superior to EVL alone.[114] Although the use of nonselective β-blockers (with or without nitrates) vs sclerotherapy or in combination with sclerotherapy has been studied,[115-117] their use has been supplanted by EVL + β-blockers. TIPS provide an effective salvage therapy for those who experience recurrent bleeding despite EVL + β-blockers.[118,119] Liver transplantation should be considered if bleeding recurs despite a patent TIPS. TIPS patency is substantially superior with coated stents, which should be used whenever possible.

Gastric varices. Gastric varices are classified as gastroesophageal varices (GOV) or isolated gastric varices. Esophageal varices that extend along the lesser and greater curves are called *GOV1* and *GOV2*, respectively.[120] GOV1 can be treated like esophageal varices. GOV2 bleed more often than GOV1 and have a higher mortality as well.[120] Isolated gastric varices commonly exist in the fundus and are often associated with spontaneous splenorenal collaterals. They bleed at lower HVPG than esophageal varices and bleed more severely.[120] There is no consensus on primary prophylaxis of bleeding for isolated gastric varices. Once bleeding occurs, both cyanoacrylate injection sclerotherapy and TIPS have been used effectively to establish hemostasis and prevent rebleeding.[121,122]

Management of Ascites

Ascites is a common complication of cirrhosis and is a marker of poor outcomes.[123-126] Although many cases are medically manageable, a fraction of subjects become refractory to medical therapy.[127] HRS often superimposes over refractory ascites. Although 50% of those with refractory ascites die within 6 months, the median survival after onset of type 1 HRS—the rapidly developing form of HRS—is 1.7 weeks.[51]

Management of uncomplicated ascites. The initial diagnostic evaluation of ascites should always include a paracentesis. A serum to ascites albumin gradient >1.1 establishes the presence of portal hypertension-related ascites. A neutrophil count >250/mm^3 is diagnostic of SBP.[128-130] The severity of ascites may vary from that only detectable by imaging studies (grade 1) to that which is clinically obvious but not tense (grade 2) and tense ascites (grade 3).[131] The goals of management of uncomplicated ascites are to provide symptoms relief, create a negative Na balance, and prevent complications of ascites. Na restriction is an important component of the treatment strategy. A low Na diet (60–90 mEq/day), equivalent to 1.5–2 g of salt/day, should be prescribed along with adequate calorie and protein intake to maintain the nutritional status of the patient.

Spironolactone inhibits distal tubular Na reabsorption by antagonizing aldosterone. The biologic effect half-life of spironolactone extends over days. It can therefore be dosed once a day, and dose changes should not be performed at less than 7-day intervals.[132] The adverse effects of spironolactone include hyponatremia, hyperkalemia, and painful gynecomastia. These may require a switch to amiloride, a less effective diuretic, which has a distinct mechanism of action and does not cause gynecomastia. The utility of canrenoate and eplerenone, which also act on the distal tubule, have not been extensively validated for cirrhotic ascites. Spironolactone has a synergistic effect with furosemide, a loop-acting diuretic that is less effective than spironolactone as a single agent for cirrhotic ascites[133] These drugs should be used in combination whenever possible.[132,134] Therapy is usually started with 100 mg/day spironolactone and 40 mg furosemide and doses modified based on either adverse effects or lack

Table 3. Typical Urinary Findings in Renal Failure in Patients With Ascites

Parameter	Osmolality mosm/kg	Urine (Na) mmol/L	Sediment	Protein mg/day
Prerenal				
Hypovolemia	>500	<20	Normal	<500
Hepatorenal syndrome	>500	<10	Normal	<500
Renal				
Acute tubular necrosis	<350	>40	Granular casts	500–1500
Interstitial nephritis	<350	>40	WBC eosinophils[a]	500–1500
Glomerular disease	Variable	Variable	Red cell casts	Often >1500

WBC, white blood cells.
[a]Often because of drugs.

of response (<1.5 kg weight loss/week). Subjects with edema can tolerate more aggressive diuresis.[135]

Large volume paracentesis (>5 L removed at a single sitting) (LVP) is used mainly for symptom relief and rapid mobilization of tense ascites.[136] LVP is sometimes associated with postparacentesis circulatory dysfunction, characterized by worsened vasodilation, hyponatremia, increased renin, and norepinephrine activity.[137] Intravenous administration of albumin (6–8 g/L ascites removed) reduces the risk of postparacentesis circulatory dysfunction, which has been associated with an increased mortality risk.[138,139] Total paracentesis can be performed safely as long as albumin is given to prevent postparacentesis circulatory dysfunction.

Management of refractory ascites. Refractory ascites (Table 1) is associated with increasing systemic vasodilation, decreased effective circulating volume, and renal perfusion.[140] Repeated LVP or total paracentesis are the most commonly used modalities for the treatment of refractory ascites. Although they immediately relieve ascites, they are associated with ascites recurrence in most subjects and do not improve survival.[141,142] TIPS decompress the hepatic sinusoids and promote an increase in central volume, thereby decreasing proximal tubular Na reabsorption and causing a natriuresis over a period of several weeks.[143] TIPS are superior to LVP for long-term control of ascites.[144,145] However, this does not translate into improved survival, and the decrease in ascites-related health care resource utilization is offset by increased encephalopathy-related morbidity.[145,146] In addition, for the same survival outcomes, TIPS is less cost-effective than LVP.[147] Hyperbilirubinemia, severe hypoprothrombinemia, and renal failure are risk factors associated with a poor outcome after TIPS.[148] The outcomes of TIPS for refractory ascites are best in those who have failed repeated LVP and have relatively preserved liver and renal function, ie, a creatinine level <1.5 mg/dL, international normalized ratio <1.5, and bilirubin level <2 mg/dL. Ideally, it should be used as a bridge to liver transplantation.

Management of hepatorenal syndrome. Type 2 HRS usually occurs in the setting of refractory ascites and is managed as refractory ascites. The use of intravenous albumin with initial antibiotic therapy for SBP decreases the risk of developing HRS and must always be given in this situation.[149] In addition, in a single trial, pentoxyfylline treatment of alcoholic hepatitis decreased the incidence of HRS.[150] It is important to recognize that HRS only accounts for 15%–20% of cases of renal insufficiency in those with cirrhosis and that hypovolemia, acute tubular necrosis, and iatrogenic renal toxicity remain important causes of renal failure in this population (Table 3).[151,152]

The initial approach to the evaluation of sudden worsening of renal function in a subject with cirrhosis includes (1) exclusion of iatrogenic or other causes of renal failure, (2) aggressive evaluation for and treatment of sepsis, and (3) excluding volume depletion by clinical assessment and a therapeutic challenge with albumin (1 g/kg or up to 100 g) given intravenously.[52] Type 1 HRS adds to the value of the Model for End-Stage Liver Disease score to predict mortality with medical treatment.[153] Liver transplantation is the only definitive treatment of HRS, and the outcomes depend on successful treatment of HRS prior to transplantation.[154,155] However, renal function may take months to recover and in some subjects may not recover at all. A variety of systemic vasoconstrictors (midodrine, ornipressin, terlipressin, and norepinephrine) have been used to reverse the systemic arterial vasodilation that drives effective hypovolemia and renal vasoconstriction in subjects with HRS.[156–158] A recent randomized placebo-controlled trial found terlipressin to be effective in reversing type 1 HRS without affecting overall survival.[159] Moreover, in those with HRS reversal, a marked improvement in survival was noted. In addition, recurrence of HRS after reversal was rare in this study. These exciting preliminary data provide hope for subjects with an otherwise fatal disease. It also provides a means to keep the patient alive while an organ is sought for transplantation.

In subjects with cirrhosis, renal failure, and severe sepsis, hydrocortisone may improve the hemodynamic abnormalities in HRS and may be used especially if a response to vasopressors is not seen.[160] Dialysis support alone does not improve long-term survival.[161]

Other ascites-related complications. Dilutional hyponatremia results from the release of antidiuretic hormone triggered by severe effective hypovolemia. It is a marker of poor outcome and predicts the development of HRS.[44,162] The initial management includes volume restric-

tion to 1500 cc/day. For serum Na levels <125 mEq/L, more severe volume restriction is recommended. However, it is difficult to comply with this limit. Preliminary data with aquaretic drugs that promote free water excretion by activating aquaporin channels in the nephron suggest that these could be an exciting class of drugs that can correct both ascites and dilutional hyponatremia.[163]

Hepatic hydrothorax results from movement of ascites across diaphragmatic fenestrae into the pleural cavity. It is initially managed by Na restriction, diuretics, and intermittent thoracentesis. TIPS have been used effectively in some patients with refractory hydrothorax.[164] Placement of an indwelling catheter in the pleural cavity in such cases is associated with infection and a very high mortality and should be avoided.

Primary prophylaxis for SBP with an oral quinolone should be considered in those with low protein ascites.[165] SBP should always be considered in the differential diagnosis when a patient with cirrhosis and ascites develops fever, abdominal pain, altered mental status, variceal hemorrhage, or azotemia. It is diagnosed by a diagnostic paracentesis and treated with a third-generation cephalosporin.[166] A 5-day course has been found to be as effective as a 10-day course for uncomplicated SBP.[167] SBP recurs frequently, and secondary prophylaxis with oral quinolones has been shown to be effective in preventing recurrence and is therefore recommended.[166,168]

Management of HE

There is a dearth of large scale, rigorously performed clinical trials evaluating the efficacy of various treatment for HE. The approach to management outlined below reflects the best evidence available and expert opinion.

Approaches for HE. *Removal of the precipitating factor.* Volume depletion and azotemia are important precipitants of HE. Diuretic-induced HE may also arise from the effects of hypokalemia and from urea-fueled ammoniagenesis. Hydration is the key therapeutic approach. In one study, albumin was more efficacious than saline in reversing diuretic-induced HE.[169] The mechanism for this effect is unclear, but the authors postulated a beneficial role for the antioxidant properties of albumin. Systemic infections can also precipitate HE and should be looked for and treated.

Reducing nitrogen and ammonia load. Diet: Prescription of low-protein diets for patients with HE should be abandoned. Even in patients admitted with an episode of HE, a randomized controlled trial showed no difference in the rate of awakening after prompt resumption of protein in the diet vs progressive increments over a 14-day period.[170] The ingestion of vegetable protein, the preferred protein source, may be limited by the acceptance of such diets in the Western world, and a consultation with a dietitian may be useful. Randomized-controlled trials have shown benefits of branched-chain amino acid supplementation on a composite outcome of time to decompensation and death.[171,172] The beneficial effects may be related to the anabolic effects of leucine.

Nonabsorbable disaccharides. Although there is a paucity of placebo controlled randomized clinical trials, there is extensive clinical experience with nonabsorbable disaccharide drugs. The mechanisms of action include acidification of the colon and a reduction in cerebral water content.[173,174] In a recent study, lactulose improved neuropsychologic function in a large cohort of Indian patients.[175]

Antibiotics. Neomycin, metronidazole, and rifaximin, which have widely different antimicrobial spectra, have been used to treat HE. A meta-analysis suggested slightly better outcomes with antibiotics compared with nonabsorbable disaccharides.[176] A recently completed study showed no differences between rifaximin and placebo in patients with minimal/mild encephalopathy[177]; a subgroup of patients with asterixis was reported to benefit from the drug. Additional studies are currently underway.

Probiotics. Probiotics, a term that includes a wide range of nonpathogenic microorganisms, have been used in a wide range of digestive disorders.[178,179] Colonization with nonurease containing lactobacilli would result in a reduction in colonic ammoniagenesis. Indeed, in a human study in which a probiotic preparation was combined with fiber in patients with cirrhosis,[178] a reduction in circulating ammonia levels was seen. In this study, positive effects on intestinal permeability were likely because circulating endotoxin levels were decreased.

Agents that increase ureagenesis. Ammonia utilization for hepatic urea synthesis can be increased by Na phenylbutyrate (which eliminates 2 nitrogen atoms by forming phenylacetylglutamine) or Na benzoate, which binds to glycine (1 nitrogen atom) and is excreted by the kidneys as hippuric acid.[180-182] Experience with these drugs in HE is limited,[181,182] but a commercial preparation that combines both agents in an intravenous formulation may undergo testing in the United States. Zinc supplementation has also been used to increase ureagenesis. Although its use is generally considered to be safe, a pathogenic role for Zn in neuronal damage in some neurologic diseases has been reported.[183] It should certainly be used if Zn deficiency is present.[184] Ornithine-aspartate provides substrate for both urea and glutamine synthesis. It accelerates the recovery from grade 2 encephalopathy and is available in an intravenous formulation outside the United States.[59]

Agents that work directly on the brain. A meta-analysis of flumazenil, a benzodiazepine receptor antagonist, indicated a beneficial effect on short-term awakening from deeper stages of encephalopathy[185]; the drug is, however, not available for chronic administration. Although the brain remains a key direct target for treatment of HE, there are no available agents that have been shown to improve HE by this mechanism.

Clinical scenarios. The different types of HE[186] require different therapeutic approaches.

Precipitant-induced encephalopathy. Removal of the precipitant is a key factor in precipitant-induced encephalopathy. The benefit of other therapies in this situation is difficult to judge because removal of the precipitant per se has a major impact in the resolution of the episode.

Persistent encephalopathy. Two types of patients present with the persistent encephalopathy form of HE. With relatively well-preserved liver function, the possibility of a large spontaneous portal-systemic shunt should be considered because improvement of HE can occur after radiologic closure. For patients with more advanced liver disease, persistent encephalopathy and recurrent episodes of encephalopathy are treated with nonabsorbable dissacharides and/or antibiotics. Other therapies may be considered as second-line approaches. Transplantation is indicated for otherwise appropriate candidates.

Minimal encephalopathy. The need to treat minimal encephalopathy is as yet unclear. In patients in whom functional impairment is present, there may be a potential for improvement.[187] Lactulose and rifaximin are often used, although data from randomized clinical trials are lacking.

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Conflicts of interest: A. J. Sanyal: Advisor to Orphan Therapeutics; ad hoc advisory board Salix; and ad hoc advisory boards of Sanofi, Gilead, Vertex, and Pfizer for nonportal hypertension-related products. A. Biel: Consultant for Salix Pharmaceuticals. J. Bosch and V. Arroyo report no conflicts of interest.

Circulatory Function and Hepatorenal Syndrome in Cirrhosis

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The pathogenic mechanism of hepatorenal syndrome is not well established. We investigated the circulatory function in cirrhosis before and after the development of hepatorenal syndrome. Systemic and hepatic hemodynamics and the activity of endogenous vasoactive systems were measured in 66 patients who had cirrhosis with tense ascites and normal serum creatinine levels; measurements were repeated at follow-up in 27 cases in whom hepatorenal syndrome had developed. At baseline, mean arterial pressure and cardiac output were significantly higher, and hepatic venous pressure gradient, plasma renin activity, and norepinephrine concentration were significantly lower in patients who did not develop hepatorenal syndrome compared with those presenting with this complication. Peripheral vascular resistance was decreased to the same extent in the two groups. Plasma renin activity and cardiac output were the only independent predictors of hepatorenal syndrome. Hepatorenal syndrome occurred in the setting of a significant reduction in mean arterial pressure (83 ± 9 to 75 ± 7 mmHg; $P < .001$), cardiac output (6.0 ± 1.2 to 5.4 ± 1.5 L/min; $P < .01$), and wegded pulmonary pressure (9.2 ± 2.6 to 7.5 ± 2.6 mmHg; $P < .001$) and an increase in plasma renin activity (9.9 ± 5.2 to 17.5 ± 11.4 ng/mL · hr; $P < .001$), norepinephrine concentration (571 ± 241 to 965 ± 502 pg/mL; $P < .001$), and hepatic venous pressure gradient. No changes were observed in peripheral vascular resistance. In conclusion, these data indicate that hepatorenal syndrome is the result of a decrease in cardiac output in the setting of a severe arterial vasodilation. (HEPATOLOGY 2005;42:439-447.)

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Hepatorenal syndrome is a functional renal failure due to intense renal vasoconstriction that frequently develops in patients with cirrhosis and ascites.[1] Two types of hepatorenal syndrome have been identified.[2] Type 1 is characterized by rapidly progressive renal failure. It frequently follows a precipitating event—usually an infection—and is associated with extremely short survival. Type 2 is characterized by moderate and steady renal failure that develops insidiously. It is usually detected in patients who respond poorly to diuretics and is associated with longer survival.

Hepatorenal syndrome occurs in the setting of a circulatory dysfunction characterized by arterial hypotension and marked activation of the renin-angiotensin and sympathetic nervous systems.[2] Because there is vasoconstriction in the kidneys[3] and in other extrasplanchnic territories,[4,5] the suggestion has been raised that hepatorenal syndrome is caused by an accentuation of the splanchnic arterial vasodilation present in nonazotemic patients with cirrhosis and ascites.[6] However, there is no study proving this contention. The potential differences in systemic hemodynamics between type 1 and type 2 hepatorenal syndrome have never been explored.

This article reports a study assessing systemic and hepatic hemodynamics and the activity of the endogenous vasoactive systems in a large series of patients who had cirrhosis with ascites before and after the development of hepatorenal syndrome.

Patients and Methods

Study Design. Patients under 75 years of age without insulin-dependent diabetes mellitus, arterial hyperten-

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sion, or any significant disease other than cirrhosis who were admitted to the hospital with tense ascites and normal serum creatinine concentration (<1.2 mg/dL) were considered for the study. Patients with tense ascites and infection or gastrointestinal hemorrhage were considered after 5 days of recovery from these complications; patients with encephalopathy were considered after 2 days of recovery provided they showed normal serum creatinine concentration at the time of resolution of these complications. Diagnosis of cirrhosis was based on histology or on clinical, laboratory, and ultrasonography findings. Complete history and physical examination, chest and abdominal X-rays, electrocardiography, abdominal ultrasonography, laboratory tests, and blood and ascitic fluid cultures were performed. Patients were excluded if they had proteinuria above 500 mg/dL, abnormal renal ultrasonography, or hepatocellular carcinoma. Patients gave written informed consent to participate in the study, which was approved by the Ethics Committee of the Hospital Ramón y Cajal and conducted according to the guidelines of Good Clinical Practice.

A baseline study was performed after at least 4 days on a 50-70 mmol/d sodium diet and without diuretics or beta-blockers. At 8 A.M. of the fifth day, after overnight fasting and following 1 hour of bed rest, samples were obtained to measure liver and renal function tests, plasma renin activity, and plasma concentrations of aldosterone and norepinephrine. Urine was subsequently collected for 24 hours. Hemodynamic measurements were performed on the sixth day. Patients were then treated by total paracentesis plus intravenous albumin (8 g/L of ascitic fluid removed; Grifols International S.A., Barcelona, Spain), discharged from the hospital with diuretics, and followed up until the end of the study (1 year after the inclusion of the last patient), liver transplantation, or death. Diuretic dosage was adjusted to prevent ascites recurrence. Patients were advised to avoid nonsteroidal anti-inflammatory drugs.

Patients who developed hepatorenal syndrome during follow-up were studied again using an identical protocol. Because of the high prevalence of prerenal azotemia in decompensated patients with cirrhosis who were treated with diuretics, and because of the complexity of the differential diagnosis of hepatorenal syndrome, which requires an acute expansion of the plasma volume, renal failure detected in otherwise uncomplicated patients during their regular follow-up visits to the outpatient clinic were considered to be diuretic-induced. Hepatorenal syndrome was therefore diagnosed in all patients during a hospital admission for the treatment of a complication: tense ascites refractory to diuretics in most patients with type 2 hepatorenal syndrome and in some with type 1 hepatorenal syndrome; encephalopathy; infection; or gastrointestinal hemorrhage. In 1 patient, hepatorenal syndrome was detected in the postoperative period of a partial hepatectomy to remove a hepatocellular carcinoma. The diagnostic criteria for hepatorenal syndrome were those proposed by the International Ascites Club.[2] In patients with bacterial infections or gastrointestinal hemorrhage and in patients with hepatic encephalopathy, the protocol was repeated after 5 and 2 days, respectively, of recovery from these complications. Ascites was treated after completion of the protocol. No patient had more than one follow-up investigation.

Hemodynamic and Neurohormonal Measurements. Under local anesthesia, a catheter introducer (USCI International, Galway, Ireland) was placed in the right jugular vein using the Seldinger technique. Under fluoroscopic guidance, a Swan-Ganz catheter (Edwards Laboratory, Los Angeles, CA) was advanced into the pulmonary artery for measurement of cardiopulmonary pressures and cardiac output via thermodilution. A 7 French balloon-tipped catheter (MediTech Cooper Scientific Corp., Watertown, MA) was advanced into the main right hepatic vein to measure wedged and free hepatic venous pressures and the hepatic venous pressure gradient. Measurements were performed in triplicate, and the average was taken.[7] The external zero-pressure point was at the level of the right atrium (midaxillary line). The hepatic blood flow was measured during a continuous intravenous infusion of an indocyanine green solution (Serb; Laboratoires Pharmaceutiques, Paris, France) at a constant rate of 0.1 or 0.2 mg/min^{-1} (Child-Turcotte-Pugh class C and B patients, respectively) as previously described.[8] A hepatic extraction of more than 10% and steady venous indocyanine green solution levels were required for the calculation of hepatic blood flow. Heart rate and arterial pressure were measured with an automatic sphyngomanometer (Dinamap-Critikion, Tampa, FL). Systemic vascular resistance was calculated as mean arterial pressure (mm Hg) − right atrial pressure (mm Hg)/cardiac output (L/min^{-1}) × 80. Stroke work was calculated as (MAP-PWCP) × (stroke volume) × 0.0136 (gm-m). Left ventricular stroke work was calculated as systolic arterial pressure × systolic volume × 0.0136 (gm-m).

Plasma renin activity and plasma concentration of aldosterone and norepinephrine were determined via radioimmunoassay (Clinical Assays, Cambridge, MA; Diagnostic and Products Corp., Los Angeles, CA; and CAIBL Laboratories, Hamburg, Germany, respectively).[9,10] Values in healthy subjects on a low sodium diet

were: 1.35 ± 0.94 ng/mL · hr, 24.2 ± 11.3 ng/dL, and 253 ± 114 pg/mL, respectively.

Statistical Analysis. Calculations were performed with SPSS version 10.0 software (SPSS, Chicago, IL). Comparisons between groups were performed with the chi-square test or Fisher exact test for categorical data and the Student *t* test and Mann-Whitney test for continuous data. Stepwise logistic regression was used to identify independent predictors for development of hepatorenal syndrome. Probability of survival curves was constructed using the Kaplan-Meier method and was compared with the log-rank test. Patients submitted to liver transplantation or who were lost from follow-up were considered censored. Results are expressed as the mean ± SD. All reported *P* values are two-tailed, with values less than .05 considered significant.

Results

Clinical Data. Eighty-two patients admitted between February 1995 and November 1999 who agreed to participate in the study were considered. Nine patients were excluded before baseline investigations because of hepatocellular carcinoma (n = 5) or renal, cardiac, or respiratory disease (n = 4). Seven additional patients were excluded after baseline investigations because they were lost from follow-up (n = 6) or refused to continue in the study (n = 1). The investigation thus included 66 patients. Forty-seven of the patients were male, and the mean age was 60 ± 9 years. The cause of cirrhosis was alcoholic in 35 patients, hepatitis C virus infection in 25, and alcohol plus hepatitis C in 6. Fifteen of the 41 patients with alcoholism were active drinkers at inclusion. Four of them stopped drinking during follow-up. On the other hand, 5 out the 26 abstainers at inclusion reassumed alcohol intake during the study period. Most of the patients were admitted to the hospital for the treatment of an episode of tense ascites alone (n = 54) or associated to hepatic encephalopathy (n = 6). The remaining 6 patients were admitted with ascites and severe infections (n = 4) or gastrointestinal hemorrhage (n = 2). In these 6 patients, arterial pressure, pulse rate, and renal function remained stable during the 5-day washout period between recovery from these complications and the initiation of the protocol. At inclusion, 34 patients were Child-Turcotte-Pugh grade C, and 32 were grade B. Five patients (2 with hepatorenal syndrome) underwent transplantation.

The 7 patients excluded after baseline measurements did not differ from the 65 included into the study regarding the cause of cirrhosis (4 had alcoholic cirrhosis and 3 had cirrhosis associated with hepatitis C) and Child-Turcotte-Pugh grade (3 were grade B and 4 were grade C). There were also no differences between patients excluded after baseline measurements and those included into the study in age, sex, renal and hepatic function, systemic and hepatic hemodynamics, and degree of activity of the renin-angiotensin and sympathetic nervous systems (data not shown).

Thirty-nine patients did not develop hepatorenal syndrome during the study period (group A). The remaining 27 patients developed hepatorenal syndrome (group B). The prevalence of hepatorenal syndrome was unrelated to the cause of cirrhosis. On the other hand, in patients with alcohol-associated cirrhosis, the prevalence of hepatorenal syndrome was similar in active drinkers (8 cases with hepatorenal syndrome from the 20 active drinkers at inclusion or during follow-up) and abstainers (7 cases with hepatorenal syndrome from the 21 patients who abstained throughout the study period). Hepatorenal syndrome was type 1 in 12 cases and type 2 in 15. Type 1 hepatorenal syndrome was chronologically related to severe bacterial infection in 6 cases and to surgical operation and variceal hemorrhage in 1 case. Type 1 hepatorenal syndrome was detected during a hospitalization for refractory ascites (n = 2) and hepatic encephalopathy (n = 3) in the 5 patients without a precipitating event. The time between baseline and follow-up studies in patients developing hepatorenal syndrome was 359 ± 212 days (275 ± 153 and 425 ± 233 in patients with type 1 and type 2 hepatorenal syndrome, respectively). The mean follow-up period in the group A patients was 639 ± 329 days.

Differences Between Patients From Groups A and B at Baseline and Changes Associated With the Development of Hepatorenal Syndrome in Patients From Group B. At baseline, patients from group A showed significantly higher mean arterial pressure, cardiac output, stroke volume, stroke work, and urinary sodium excretion and significantly lower plasma renin activity, plasma concentrations of aldosterone and norepinephrine, wedged hepatic venous pressure, and hepatic venous pressure gradient compared with patients from group B (Table 1). Although baseline serum creatinine was within the normal limits in all cases, the mean value was significantly lower in group A. There were no differences in peripheral vascular resistance and heart rate. Of the 10 variables showing significant difference between groups, only plasma renin activity (RR: 31.3; 95% CI: 6.5-150.3; $P < .0001$) and cardiac output (RR: 5.8; 95% CI: 1.3-25.2; $P < .05$) were independently associated with the development of hepatorenal syndrome according to a multivariate analysis (Fig. 1).

Development of hepatorenal syndrome in group B was associated with a significant decrease in prothrombin in-

Table 1. Baseline Measurements in Patients Who Did Not Develop Hepatorenal Syndrome (Group A) and Baseline and Follow-up Measurements in Patients Who Presented With Hepatorenal Syndrome (Group B)

	Group A (n = 39)	Group B (n = 27)	
	Baseline Measurements	Baseline Measurements	Follow-up Measurements
Serum bilirubin (mg/dL)	2.7 ± 1.9	3.8 ± 3.9	4.3 ± 3.9
Serum albumin (g/L)	24 ± 4	24 ± 5	24 ± 4
Prothrombin index (%)	64 ± 14	59 ± 14	51 ± 13††††
Child-Turcotte-Pugh score (points)	9.7 ± 1.3	9.9 ± 1.3	10.8 ± 2.1†
MELD score (points)	13.7 ± 4.0	15.8 ± 4.6	25.7 ± 6.8††††
Serum creatinine (mg/dL)	0.85 ± 0.18	1.05 ± 0.26***	3.03 ± 1.49††††
Serum sodium (mmol/L)	134.5 ± 4.8	132.6 ± 4.6	127.0 ± 5.1††††
Urinary sodium (mmol/L)	17.4 ± 18.9	7.0 ± 6.1***	4.0 ± 4.5†
MAP (mmHg)	88 ± 9	83 ± 9*	75 ± 7††††
HR (bpm)	87 ± 15	85 ± 13	82 ± 14
RAP (mmHg)	6.7 ± 2.5	6.9 ± 2.6	5.7 ± 2.2†
PAP (mmHg)	15.2 ± 3.8	14.3 ± 4.3	12.8 ± 2.8††
PCWP (mmHg)	9.2 ± 3.2	9.2 ± 2.6	7.5 ± 2.6††††
CO (L/min)	7.2 ± 1.8	6.0 ± 1.2**	5.4 ± 1.5†††
SVR (dyne · s/cm^{-5})	962.0 ± 256.4	1,058.6 ± 265.6	1,096.1 ± 327.6
Stroke volume (mL/beat)	85.2 ± 17.0	73.2 ± 18.9*	65.3 ± 18.8†
Stroke work (gm-m)	91.3 ± 17.9	75.3 ± 22.9**	62.7 ± 21.3††††
Left ventricular stroke work (gm-m)	140.0 ± 32.6	114.2 ± 43.5*	88.5 ± 32.3††††
Plasma renin activity (ng/mL · hr)	3.1 ± 2.3	9.9 ± 5.2****	17.5 ± 11.4††††
Plasma aldosterone (ng/dL)	32.0 ± 30.7	130.5 ± 69.4****	202.5 ± 130.0††††
Plasma norepinephrine (pg/mL)	221.6 ± 68.2	571.1 ± 241.1****	965.0 ± 502.5††††
WHVP (mmHg)	28.0 ± 4.0	30.5 ± 4.0*	29.5 ± 5.0
FHVP (mmHg)	11.5 ± 3.0	11.0 ± 4.0	8.5 ± 3.5††
HVPG (mmHg)	16.5 ± 3.0	19.5 ± 3.0***	21.0 ± 4.0††
HBF (mL/min)‡	1,123 ± 328.0	948 ± 221.1	713 ± 188.4††††

NOTE. Data are presented as mean ± SD.

Abbreviations: MELD, Model for End-Stage Liver Disease; MAP, mean arterial pressure; HR, heart rate; RAP, right atrial pressure; PAP, pulmonary artery pressure; PCWP, pulmonary capillary wedged pressure; CO, cardiac output; SVR, systemic vascular resistance; WHVP, wedged hepatic venous pressure; FHVP, free hepatic venous pressure; HVPG, hepatic venous pressure gradient; HBF, hepatic blood flow.

*$P < .05$; **$P < .01$; ***$P < .005$; ****$P < .001$ with respect to baseline values of group A.

†$P < .05$; ††$P < .01$; †††$P < .005$; ††††$P < .001$ with respect to baseline values of group B.

‡A hepatic extraction greater than 10% was required for the calculation of hepatic blood flow in 15 patients of group A and 19 patients of group B.

dex; an increase in Child-Turcotte-Pugh score, Model for End-Stage Liver Disease score, and hepatic venous pressure gradient; a reduction in hepatic blood flow; dilutional hyponatremia; a significant decrease in mean arterial pressure, cardiac output, stroke volume, stroke work, and cardiopulmonary pressures (pulmonary capillary wedged pressure, pulmonary artery pressure, and right atrial pressure); and marked stimulation of the renin-angiotensin-aldosterone system and sympathetic nervous system (Table 1; Figs. 2, 3). No significant changes were observed in heart rate and peripheral vascular resistance (Table 1; Fig. 3).

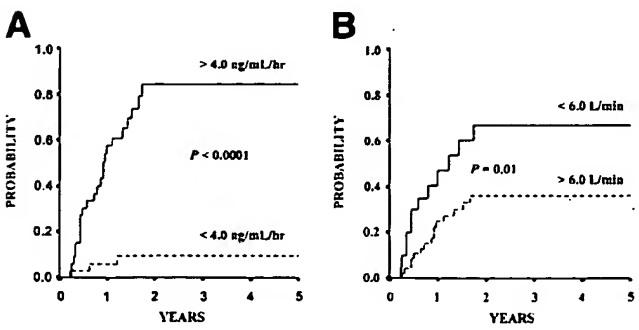

Fig. 1. (A) Probability of developing hepatorenal syndrome during follow-up in patients with baseline plasma renin activity equal or lower and higher than 4 ng/mL · hr (upper value in healthy subjects on a 50-mEq sodium diet during 5 days). (B) Probability of developing hepatorenal syndrome during follow-up in patients with baseline cardiac output higher and lower than 6 L/min (median value in the entire series of patients).

Differences Between Patients With Type 1 and Type 2 Hepatorenal Syndrome. The only significant difference between patients who developed type 1 and type 2 hepatorenal syndrome at baseline was a higher plasma renin activity and higher aldosterone and norepinephrine concentration ($P < .001$) in the former group of patients (Tables 2, 3).

Renal failure in type 2 hepatorenal syndrome was moderate in most patients (Table 2), and associated with discrete hyponatremia, a significant decrease in mean arterial pressure, and a significant increase in the degree of activity of the renin-aldosterone and sympathetic nervous systems. Cardiac output, stroke volume, stroke work, and

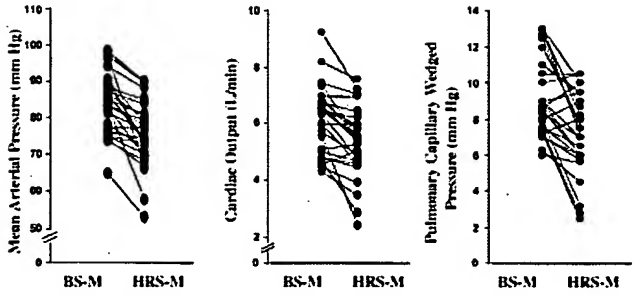

Fig. 2. Individual changes of mean arterial pressure, cardiac output, and pulmonary capillary wedged pressure associated with hepatorenal syndrome in patients from group B. BS-M, baseline measurements; HRS-M, measurements after development of hepatorenal syndrome.

cardiopulmonary pressures decreased, although differences were not significant. No significant changes were observed in liver function and hepatic hemodynamics, with the exception of a significant decrease in hepatic blood flow. In contrast, renal failure in patients with type 1 hepatorenal failure was severe and was associated with profound hyponatremia; a marked decrease in mean arterial pressure, cardiac output, stroke volume, and stroke work; a significant reduction in cardiopulmonary pressures; intense stimulation of the renin-aldosterone and sympathetic nervous systems; impairment in hepatic function; a decrease in free hepatic venous pressure and hepatic blood flow; and an increase in wedged hepatic venous pressure gradient (Table 3). Changes associated with type 1 hepatorenal syndrome were similar in patients with and without precipitating events or in patients with and without severe bacterial infections (data not shown).

At follow-up, patients with type 1 hepatorenal syndrome showed significantly higher serum creatinine levels, Child-Turcotte-Pugh score, and Model for End-Stage Liver Disease score; lower serum sodium concentration, mean arterial pressure, cardiac output, stroke volume, and stroke work; greater stimulation of the renin-aldosterone and sympathetic nervous systems; higher wedged hepatic venous pressure and hepatic venous pressure gradient; and lower hepatic blood flow compared with patients with type 2 hepatorenal syndrome (Tables 2, 3). Significant differences were not found in peripheral vascular resistance, heart rate, and cardiopulmonary pressures. The probability of survival after diagnosis of hepatorenal syndrome was lower in patients with type 1 hepatorenal syndrome (Fig. 4).

Discussion

The current concept of the pathogenesis of circulatory and renal dysfunction in cirrhosis is based on the peripheral arterial vasodilation hypothesis. It proposes that the initial mechanism is the splanchnic arterial vasodilation that develops in these patients as a consequence of portal hypertension.[11] At the early stages of disease, there is a homeostatic increase in the cardiac output as a result of the decrease in cardiac afterload and the stimulation of the sympathetic nervous activity, leading to the characteristic hyperdynamic circulation of cirrhosis. However, as the disease progresses and splanchnic arterial vasodilation becomes more intense, this increase in cardiac output is not sufficient to maintain circulatory homeostasis. Patients then develop arterial hypotension; baroreceptor-mediated stimulation of the sympathetic nervous system, renin-angiotensin system, and antidiuretic hormone; renal sodium and water retention; and ascites. Hepatorenal syndrome is the extreme expression of this circulatory dysfunction and occurs in the setting of an intense stimulation of these endogenous vasoconstrictor systems that overcomes the compensatory effect of renal vasodilatory substances (*e.g.*, prostaglandins, nitric oxide).[6]

Type 1 and type 2 hepatorenal syndrome are clinically different. Type 2 develops insidiously in patients with advanced cirrhosis and ascites. Circulatory function and hepatic and renal function in these patients remain steady for months. Their main clinical problem is refractory ascites.[12] In contrast, the onset of type 1 hepatorenal syndrome is acute and, in most cases, associated with a precipitating event, usually an infection. In type 1 hepatorenal syndrome there is rapid deterioration of circulatory, renal, and hepatic function. The patient dies within days or weeks after the onset of the syndrome with arterial hypotension, severe renal failure, jaundice, coagulopathy, and hepatic encephalopathy. Despite these differences, both types of hepatorenal syndrome are currently consid-

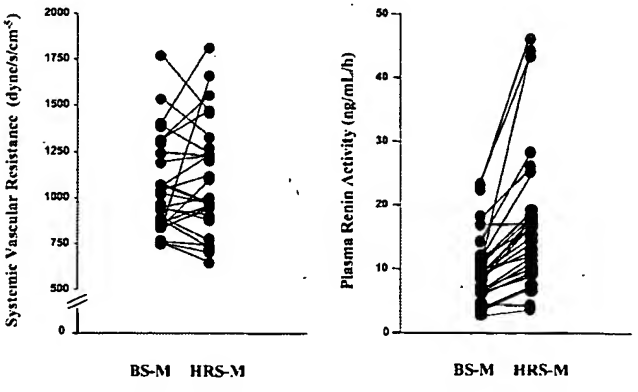

Fig. 3. Individual changes in systemic vascular resistance and plasma renin activity associated with hepatorenal syndrome in patients from group B. Systemic vascular resistance remained unchanged despite a marked increase in plasma renin activity, suggesting an accentuation of the arterial vasodilatation already present in nonazotemic cirrhosis with ascites compensated by a stimulation of the renin-angiotensin system and other endogenous vasoconstrictors. BS-M, baseline measurements; HRS-M, measurements after development of hepatorenal syndrome.

Table 2. Baseline and Follow-up Measurements In Patients Who Developed Type 2 Hepatorenal Syndrome

	Baseline Measurements (n = 15)	Follow-up Measurements (n = 15)	*P* Value
Serum bilirubin (mg/dL)	3.7 ± 4.6	3.2 ± 3.0	NS
Serum albumin (g/L)	26 ± 5	26 ± 4	NS
Prothrombin index (%)	59 ± 13	55 ± 14	NS
Child-Turcotte-Pugh score (points)	9.8 ± 1.6	9.8 ± 1.9	NS
MELD score (points)	15.6 ± 4.9	21.9 ± 5.7	<.001
Serum creatinine (mg/dL)	1.05 ± 0.2	2.11 ± 0.4	<.001
Serum sodium (mmol/L)	133.2 ± 4.7	129.6 ± 4.0	.01
Urinary sodium (mmol/L)	7.2 ± 6.2	5.6 ± 5.1	NS
MAP (mmHg)	86 ± 10	79 ± 7	.005
HR (bpm)	84 ± 12	80 ± 14	NS
RAP (mmHg)	6.8 ± 2.1	6.1 ± 1.8	NS
PAP (mmHg)	14.0 ± 3.0	12.9 ± 2.1	NS
PCWP (mmHg)	8.9 ± 1.6	8.3 ± 2.0	NS
CO (L/min)	6.2 ± 1.4	5.8 ± 1.2	NS
SVR (dyne · s/cm^{-5})	1,032.0 ± 251.3	1,014.3 ± 276.4	NS
Stroke volume (mL/beat)	75.6 ± 19.8	71.7 ± 18.2	NS
Stroke work (gm-m)	79.5 ± 28.1	73.9 ± 20.3	NS
Left ventricular stroke work (gm-m)	120.8 ± 53.5	106.5 ± 29.9	NS
Plasma renin activity (ng/mL · hr)	7.5 ± 3.7	11.9 ± 4.8	<.001
Plasma aldosterone (ng/dL)	86.8 ± 61.3	118.4 ± 80.1	.01
Plasma norepinephrine (pg/mL)	411.8 ± 155.4	628.8 ± 320.3	<.01
WHVP (mmHg)	29.5 ± 5.5	27.5 ± 5.5	<.005
FHVP (mmHg)	10.5 ± 4.0	8.0 ± 3.0	<.005
HVPG (mmHg)	19.0 ± 3.2	19.5 ± 2.0	NS
HBF (mL/min)*	1,064 ± 223	824 ± 180	<.005

NOTE. Data are presented as mean ± SD.

Abbreviations: NS, not significant; MELD, Model for End-Stage Liver Disease; MAP, mean arterial pressure; HR, heart rate; RAP, right atrial pressure; PAP, pulmonary artery pressure; PCWP, pulmonary capillary wedged pressure; CO, cardiac output; SVR, systemic vascular resistance; WHVP, wedged hepatic venous pressure; FHVP, free hepatic venous pressure; HVPG, hepatic venous pressure gradient; HBF, hepatic blood flow.

*A hepatic extraction greater than 10% was required for the calculation of hepatic blood flow in 10 patients.

ered distinct expressions of a common underlying disorder. Type 2 hepatorenal syndrome is a consequence of the natural course of the disease with a slow progression of hepatic failure, portal hypertension, and circulatory dysfunction. In type 1 hepatorenal syndrome, however, the acute effect of the precipitating event leads to rapid deterioration of circulatory function, renal and hepatic failure, encephalopathy, and death.

Many of these proposals are supported by this study. First, in baseline conditions, patients from group B were clearly at a more advanced stage of disease than patients from group A, as indicated by higher portal pressure and lower arterial pressure and urinary sodium excretion. Total peripheral vascular resistance was decreased to the same extent in the two groups, despite the existence of marked differences in the degree of stimulation of the renin-angiotensin and sympathetic nervous systems, which was higher in group B than in group A. This suggests a more intense arterial vasodilation in group B obscured by a homeostatic activation of the endogenous vasoconstrictor systems. Renal, muscular, cutaneous, and cerebral vascular resistance are increased in decompensated cirrhosis and correlate directly with the plasma levels of renin and norepinephrine.[3-5] The most likely explanation of our findings, therefore, is a progression of splanchnic arterial vasodilation before the development of hepatorenal syndrome, which does not translate into the total systemic vascular resistance because of vasoconstriction in extrasplanchnic vascular territories. Second, the development of hepatorenal syndrome in group B was associated with a decrease in mean arterial pressure and stimulation of the renin-angiotensin and sympathetic nervous systems in the absence of changes in total peripheral vascular resistance supporting a further progression of splanchnic arterial vasodilation. Finally, baseline portal pressure was higher in group B than in group A, and the development of hepatorenal syndrome in the former group of patients was associated with a further increase in portal pressure, supporting the proposal that impairment in circulatory function in cirrhosis parallels the progression of portal hypertension.

Two unexpected findings were observed in our patients, however. The first is that baseline cardiac output was significantly lower in group B than in group A. The second is that development of hepatorenal syndrome in the former group of patients occurred in the setting of a further reduction in cardiac output. These observations indicate that the progression of circulatory dysfunction in

Table 3. Baseline and Follow-up Measurements in Patients Who Developed Type 1 Hepatorenal Syndrome

	Baseline Measurements (n = 12)	Follow-up Measurements (n = 12)	P Value
Serum bilirubin (mg/dL)	3.8 ± 2.8	5.6 ± 4.5	<.05
Serum albumin (g/L)	23 ± 4	22 ± 4	NS
Prothrombin index (%)	59 ± 15	46 ± 10	<.05
Child-Turcotte-Pugh score (points)	10.1 ± 0.9	12.0 ± 1.7**	<.005
MELD score (points)	16.0 ± 4.5	30.4 ± 5.1****	<.001
Serum creatinine (mg/dL)	1.04 ± 0.3	4.26 ± 1.4****	<.001
Serum sodium (mmol/L)	132.0 ± 4.6	124.0 ± 4.6***	<.005
Urinary sodium (mmol/L)	6.9 ± 6.3	1.8 ± 2.7*	.01
MAP (mmHg)	84 ± 9	70 ± 8**	<.001
HR (bpm)	86 ± 16	84 ± 14	NS
RAP (mmHg)	7.0 ± 3.0	5.0 ± 2.0	.01
PAP (mmHg)	15.5 ± 5.5	11.5 ± 2.5	<.05
PCWP (mmHg)	9.0 ± 2.5	6.0 ± 2.5	<.001
CO (L/min)	5.8 ± 0.9	4.6 ± 1.3*	<.01
SVR (dyne · s/cm^{-5})	1099.3 ± 279.5	1211.7 ± 346.7	NS
Stroke volume (mL/beat)	70.1 ± 18.0	55.5 ± 12.1***	<.01
Stroke work (gm-m)	70.0 ± 13.5	48.8 ± 13.1***	<.005
Left ventricular stroke work (gm-m)	105.7 ± 26.2	66.0 ± 18.1****	<.005
Plasma renin activity (ng/mL·hr)	12.9 ± 5.3****	25.8 ± 12.0***	<.005
Plasma aldosterone (ng/dL)	181.4 ± 43.3****	304.5 ± 107.1	<.005
Plasma norepinephrine (pg/mL)	735.7 ± 242.0****	1,384.9 ± 346.2****	<.001
WHVP (mmHg)	32.5 ± 4.5	31.5 ± 4.5*	NS
FHVP (mmHg)	12.5 ± 4.0	9.0 ± 4.5	<.05
HVPG (mmHg)	20.0 ± 0.5	22.5 ± 0.2*	<.01
HBF (mL/min)†	818 ± 135	589 ± 103***	<.05

NOTE. Data are presented as mean ± SD.

Abbreviations: NS, not significant; MELD, Model for End-Stage Liver Disease; MAP, mean arterial pressure; HR, heart rate; RAP, right atrial pressure; PAP, pulmonary artery pressure; PCWP, pulmonary capillary wedged pressure; CO, cardiac output; SVR, systemic vascular resistance; WHVP, wedged hepatic venous pressure; FHVP, free hepatic venous pressure; HVPG, hepatic venous pressure gradient; HBF, hepatic blood flow.

*$P < .05$; **$P < .01$; ***$P < .005$; ****$P < .001$ with respect to values (baseline and follow-up) of patients who developed type 2 hepatorenal syndrome shown in Table 2.

†A hepatic extraction greater than 10% was required for the calculation of hepatic blood flow in 9 patients.

cirrhosis is not only due to an accentuation of the splanchnic arterial vasodilation, which is the proposal of the peripheral arterial vasodilation hypothesis, but also to a reduction in cardiac output. The decrease in cardiopulmonary pressures observed in group B patients suggests a reduction in cardiac preload as an important mechanism of the fall in cardiac output. An impaired chronotropic function is also a contributory mechanism. In fact, heart rate in baseline conditions was similar in patients in both groups, despite important differences in the degree of stimulation of the sympathetic nervous system. Moreover, in patients developing hepatorenal syndrome, no change in heart rate was observed, despite a marked deterioration in circulatory function and an increase in sympathetic nervous activity. There are studies supporting the existence of a cirrhosis-related cardiomyopathy associated with an impairment in left ventricular function,[13-16] which may also play a role in the reduction in cardiac output.

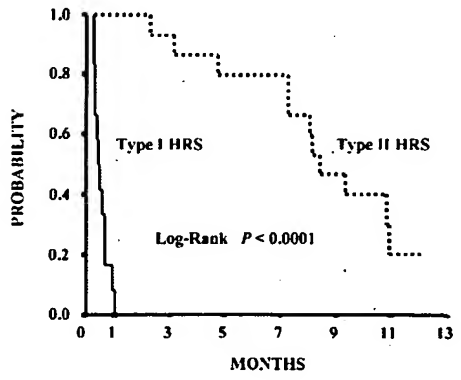

Fig. 4. Probability of survival after the diagnosis of hepatorenal syndrome (HRS). Patients are grouped according to the type of hepatorenal syndrome.

Our data suggest that this decrease in cardiac output is a very relevant event in the clinical course of cirrhosis, because it is an independent predictor of hepatorenal syndrome, the most important prognostic factor in patients with decompensated cirrhosis. This feature is not surprising. Following impairment in cardiac output, arterial pressure homeostasis is solely dependent on the activity of the renin-angiotensin system, sympathetic nervous sys-

tem, and antidiuretic hormone, which have detrimental effects on the renal circulation and probably also in the circulation of other organs. Previous studies have shown that cutaneous and muscular blood flow are markedly decreased and cerebral vascular resistance is increased in patients with hepatorenal syndrome.[3-5] In the current study, we have observed that hepatorenal syndrome is also associated with an intense reduction in hepatic blood flow. Vasoconstriction and reduction of blood flow to essential organs such as the kidneys, brain, and liver may offer a rational explanation of many features observed in patients with hepatorenal syndrome, including the deterioration in renal and hepatic function and the development of encephalopathy.

Changes in mean arterial pressure and peripheral vascular resistance and in the activity of the renin-angiotensin system and sympathetic nervous system were qualitatively similar in patients developing type 1 and type 2 hepatorenal syndrome, although the intensity of the changes was higher in the former group of patients. This is consistent with the concept that the two types of hepatorenal syndrome develop as a consequence of an accentuation of the arterial vasodilation already present in nonazotemic cirrhosis with ascites. In patients with type 1 hepatorenal syndrome, arterial hypotension and the stimulation of the endogenous vasoconstrictor systems occurred in the setting of a significant decrease in cardiac output and cardiopulmonary pressures. In contrast, no significant changes in these parameters were observed in patients with type 2 hepatorenal syndrome. These data indicate that circulatory dysfunction in patients with type 1 hepatorenal syndrome is related to the simultaneous occurrence of an increase in the degree of arterial vasodilation and a reduction in cardiac output, whereas it would be related only to an accentuation of arterial vasodilation in type 2 hepatorenal syndrome. This could account for the greater severity of circulatory dysfunction in type 1 hepatorenal syndrome. Nevertheless, our data also indicate that cardiac function in patients with type 2 hepatorenal syndrome is normal. Although not significantly, cardiac output decreased in these patients, whereas it would be expected to increase owing to the reduction in arterial pressure. Moreover, the impairment in chronotropic function was comparable in patients with type 1 and type 2 hepatorenal syndrome. The mechanism of the reduction in cardiopulmonary pressures in type 1 hepatorenal syndrome can not be ascertained from our data. It could be related to a decrease in plasma volume, an increase in venous compliance, or both. Further studies assessing cardiovascular hemodynamics in hepatorenal syndrome are needed to clarify these features.

The combined administration of intravenous albumin and vasoconstrictors (*e.g.*, terlipressin[17,18] and alfa-1 agonists[19-21]) normalizes circulatory function and serum creatinine in most patients with type-1 hepatorenal syndrome. These effects, however, are rarely obtained when vasoconstrictors or intravenous albumin are given alone.[22] In contrast, the intravenous administration of albumin alone is highly effective in the prevention of circulatory dysfunction and type 1 hepatorenal syndrome in patients with spontaneous bacterial peritonitis.[23] Our study offers a rational explanation for these features. The increase in cardiac preload that follows intravenous albumin administration increases cardiac output and eliminates one of the pathogenic factors of the circulatory dysfunction in decompensated cirrhosis. This would be sufficient to prevent hepatorenal syndrome. However, when renal failure is already established, correction of the two pathogenic factors—the splanchnic arterial vasodilation and the impaired cardiac output—is required to reverse the intense circulatory dysfunction and renal vasoconstriction associated with type 1 hepatorenal syndrome.

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ORIGINAL ARTICLE

Beta-Blockers to Prevent Gastroesophageal Varices in Patients with Cirrhosis

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ABSTRACT

BACKGROUND

Nonselective beta-adrenergic blockers decrease portal pressure and prevent variceal hemorrhage. Their effectiveness in preventing varices is unknown.

METHODS

We randomly assigned 213 patients with cirrhosis and portal hypertension (minimal hepatic venous pressure gradient [HVPG] of 6 mm Hg) to receive timolol, a nonselective beta-blocker (108 patients), or placebo (105 patients). The primary end point was the development of gastroesophageal varices or variceal hemorrhage. Endoscopy and HVPG measurements were repeated yearly.

RESULTS

During a median follow-up of 54.9 months, the rate of the primary end point did not differ significantly between the timolol group and the placebo group (39 percent and 40 percent, respectively; P=0.89), nor were there significant differences in the rates of ascites, encephalopathy, liver transplantation, or death. Serious adverse events were more common among patients in the timolol group than among those in the placebo group (18 percent vs. 6 percent, P=0.006). Varices developed less frequently among patients with a baseline HVPG of less than 10 mm Hg and among those in whom the HVPG decreased by more than 10 percent at one year and more frequently among those in whom the HVPG increased by more than 10 percent at one year.

CONCLUSIONS

Nonselective beta-blockers are ineffective in preventing varices in unselected patients with cirrhosis and portal hypertension and are associated with an increased number of adverse events. (ClinicalTrials.gov number, NCT00006398.)

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Nonselective beta-adrenergic blockers reduce portal pressure through a reduction in portal venous inflow[1,2] as a result of a decrease in cardiac output (β_1-adrenergic blockade) and splanchnic blood flow (β_2-adrenergic blockade). Randomized, controlled trials have demonstrated that nonselective beta-blockers prevent variceal hemorrhage in patients with varices.[3] Decreasing portal pressure at earlier stages may prevent gastroesophageal varices. In fact, an experimental study demonstrated that beta-blockers prevent the development of portosystemic collateral vessels.[4] Therefore, we conducted a study to evaluate the efficacy of nonselective beta-blockers in preventing gastroesophageal varices and to assess whether baseline and sequential measurements of the hepatic venous pressure gradient (HVPG) are useful in predicting the development of varices.

METHODS

The study was an investigator-initiated, randomized, double-blind, placebo-controlled, clinical trial conducted at four sites. The protocol was approved by the institutional review board at each site, and all patients gave written informed consent. Timolol maleate (Blocadren) and placebo were provided by Merck; Merck did not participate in any other aspect of the study, including study design, data analysis, and manuscript preparation.

PATIENTS

Patients were enrolled between August 1993 and March 1999 and followed until September 2002. Eligible patients had cirrhosis and portal hypertension, as defined by an HVPG of at least 6 mm Hg; did not have gastroesophageal varices; and were older than 18 years and younger than 75 years of age. The diagnosis of cirrhosis was either biopsy-proven or clinically suspected and confirmed by the finding of an HVPG of 10 mm Hg or greater. The absence of gastroesophageal varices was determined unanimously at endoscopy by two staff endoscopists who were present during the entire procedure and who evaluated the procedure independently. Exclusion criteria included ascites requiring diuretics, hepatocellular carcinoma, splenic- or portal-vein thrombosis, concurrent illnesses expected to decrease life expectancy to less than one year, the use of any drug or procedure affecting splanchnic hemodynamics or portal pressure, primary biliary cirrhosis or primary sclerosing cholangitis, contraindications to beta-blocker therapy, pregnancy, or alcohol intake during the dose-titration phase.

Of 780 patients screened for varices, 490 (63 percent) had none. Of these 490 patients, 213 (43 percent) were included in the study. The remaining 277 were excluded for the following reasons: 92 declined to participate, 79 had concomitant illnesses, 52 had a normal HVPG (less than 6 mm Hg), 21 could not tolerate the lowest dose of timolol, 15 had an HVPG of less than 10 mm Hg and non–biopsy-proven cirrhosis, 6 were lost to follow-up, 4 were consuming alcohol during the titration phase, 4 were receiving treatment with interferon or phlebotomy, 2 had primary biliary cirrhosis, and in 2, efforts to measure the HVPG were unsuccessful.

TITRATION OF THE DOSE

The dose of timolol (or placebo) to be used during the study was determined for each patient before randomization during a titration period in which open-label timolol was administered orally. The starting dose of timolol was 5 mg per day and was increased by 5 mg every three days until one of the following occurred: the resting heart rate was reduced by 25 percent from the baseline value, the resting heart rate fell below 55 beats per minute, a daily dose of 80 mg of timolol was reached, or the patient could not tolerate a further increase in the dose.

RANDOMIZATION

After the titration period, patients were randomly assigned to receive timolol or an identical-appearing placebo tablet. The randomization code was generated by computer for each participating center. Patients were stratified according to the cause of cirrhosis (alcoholic vs. nonalcoholic) and baseline HVPG (less than 10 mm Hg vs. 10 mm Hg or more). An alcoholic cause was defined as a long-standing history of alcohol ingestion exceeding 60 g per day. In patients with a dual alcoholic and viral cause, the classification of cirrhosis was based on the clinical and histologic findings.

FOLLOW-UP

Patients were assessed clinically at baseline, one and three months after randomization, and every three months thereafter. At each visit, the heart rate, pill count, occurrence of adverse events, and alcohol consumption were determined and blood was obtained for hematologic and biochemical measurements. To maintain study blinding, the patient's

Table 1. Baseline Characteristics of the Patients.*

Characteristic	Timolol Group (N=108)	Placebo Group (N=105)
Age — yr	46±11	44±11
Male sex — no. (%)	70 (65)	56 (53)
Race or ethnic group — no. (%)		
White	100 (93)	98 (93)
Black	3 (3)	1 (1)
Latin-American	3 (3)	2 (2)
Indian	2 (2)	3 (3)
Mideastern or Arabian	0	1 (1)
Cause of cirrhosis — no. (%)		
Alcoholic†	26 (24)	25 (24)
Nonalcoholic	82 (76)	80 (76)
HCV	67 (62)	67 (64)
HBV	6 (6)	2 (2)
Cryptogenic	5 (5)	5 (5)
Other	4 (4)	6 (6)
Anti-HCV positivity — no. (%)	70 (65)	74 (70)
Child–Pugh score‡	5.4±0.7	5.4±0.8
Child–Pugh class — no. (%)		
A	98 (91)	91 (87)
B	10 (9)	14 (13)
Mean blood pressure — mm Hg	94±10	93±12
Heart rate — beats/min	75±11	74±10
Hemoglobin — g/dl	13.8±1.5	13.5±1.7
White-cell count — $\times 10^{-3}$/mm^3	5.7±2.8	5.7±2.1
Platelet count — $\times 10^{-3}$/mm^3	122±72	119±46
Total bilirubin — mg/dl	1.2±0.7	1.12±0.8
Albumin — g/dl	3.9±0.5	3.9±0.5
Prothrombin time — INR	1.34±2.53	1.34±2.52
Aspartate aminotransferase — U/liter	93±74	89±59
Alanine aminotransferase — U/liter	106±101	105±96
Alkaline phosphatase — U/liter	138±70	153±97
Serum sodium — mmol/iter	140±3	140±4
Blood urea nitrogen — mg/dl	14±8	15±10
Creatinine — mg/dl	0.9±0.2	0.9±0.2
HVPG — mm Hg	11.7±4.3	11.7±4.1
HVPG ≥10 mm Hg — no. (%)	67 (62)	67 (64)
Median follow-up — mo	52.7	57.9

* Plus–minus values are means ±SD. There were no significant differences between groups. Race or ethnic group was self-reported. To convert values for bilirubin to micromoles per liter, multiply by 17.1. To convert values for blood urea nitrogen to micromoles per liter, multiply by 0.357. To convert values for creatinine to micromoles per liter, multiply by 88.4. HCV denotes hepatitis C virus, HBV hepatitis B virus, and INR international normalized ratio.

† The majority (37 of 51 [73 percent]) had been abstinent from alcohol for more than one month; 5 others had evidence of mild alcoholic hepatitis at randomization (2 in the timolol group and 3 in the placebo group).

‡ The Child–Pugh score can range from 5 to 15, with higher scores indicating more severe liver disease.

heart rate was measured by the study nurse and not by the investigators. At baseline and every year thereafter, upper endoscopy was performed and HVPG was measured as described elsewhere.[5] According to standard practice at the time, no patient received antiviral therapy during the study.

END POINTS

The primary end points were the development of varices or variceal hemorrhage as identified unanimously at endoscopy by two staff endoscopists who were present during the entire procedure and who evaluated the procedure independently. Varices were defined by the presence of one of the following: large varices (at least 5 mm); small varices (less than 5 mm), confirmed by endoscopy 6 months later; small varices (less than 5 mm) on one endoscopy, with the patient's declining to undergo comfirmatory endoscopy or an inability to perform confirmatory endoscopy in the subsequent 12 months; or gastric varices confirmed by endoscopic ultrasonography. Variceal hemorrhage was defined as any hematemesis or melena in a patient in whom endoscopy showed active bleeding from an esophageal or gastric varix, an esophageal or gastric varix with an adherent clot, or varices but no other source of bleeding. In addition, acute, clinically significant bleeding as a result of portal hypertensive gastropathy (defined by the need for a 2-unit transfusion, a 6-point drop in the hematocrit, or a drop of more than 20 mm Hg in systolic blood pressure with a change in the patient's posture) was considered a primary end point.

Secondary end points were the development of ascites or encephalopathy, liver transplantation, or death. Data collection was terminated and treatment was considered to have failed when a patient reached the primary end point, underwent liver transplantation, or died.

ADVERSE EVENTS

An adverse event was any event that required a diagnostic or therapeutic intervention. All adverse events, regardless of their possible association with the disease or study treatment, were recorded. An adverse event was judged severe if it was considered to endanger the health or safety of the patient.

DATA AND SAFETY MONITORING BOARD

Members of a data and safety monitoring board were appointed by the National Institute of Diabetes and Digestive and Kidney Diseases and met ev-

ery six months to review the progress of the study and accumulated data. According to the protocol, one interim analysis was performed on October 26, 2000, after all patients had been enrolled. At that time, the data and safety monitoring board was empowered to recommend termination of the study on the basis of concern about safety or in the presence of sufficient evidence to indicate that timolol was statistically superior to placebo. The board voted unanimously to recommend continuation of the trial.

STATISTICAL ANALYSIS

We estimated that treatment with timolol would reduce the four-year cumulative probability of varices from 50 percent (the rate without treatment)[6,7] to 30 percent, given a statistical power of 80 percent to detect an absolute difference of 20 percent between the placebo and timolol groups at a two-sided alpha level of 0.05. We estimated that the study would require 193 patients, and we then increased this amount by 10 percent to account for the loss of patients to follow-up, yielding a total of 212 patients.

All analyses were conducted according to the intention-to-treat principle. Qualitative variables were compared by means of Fisher's exact test. Wilcoxon's rank-sum test was used to compare continuous variables or ordinal data. Actuarial probabilities were calculated according to the Kaplan–Meier method and compared with use of the log-rank test. Data were censored when the primary end point was reached, at the time of transplantation or death, or at the time of the last visit, whichever occurred first. A Cox proportional-hazards model was used to identify the variables that best explained the variability in the rates of primary end points, treatment failure, and survival. Calculations were performed with the use of the SAS statistical software package.

Table 2. Rates of Primary and Secondary End Points and Treatment Failure.

Variable	Timolol Group (N=108)	Placebo Group (N=105)	P Value
	no./total no. (%)		
Primary end point*	42/108 (39)	42/105 (40)	0.89
Large varices	4	4	
Confirmed small varices	27	30	
Unconfirmed small varices	8	5	
Variceal hemorrhage	2	3	
Hemorrhage from portal hypertensive gastropathy	1	0	
Secondary end point†	22/66 (33)	22/63 (35)	1.00
Ascites	4	6	
Death	3	2	
Hepatic encephalopathy	3	2	
Transplantation	1	0	
Death	0	1	
Ascites and encephalopathy	6	5	
Transplantation	1	0	
Death	5	5	
Transplantation	7‡	2§	
Death	10¶	15‖	
Treatment failure**	59/108 (55)	59/105 (56)	0.89

* No patient had isolated gastric varices as a primary end point. Among the patients in whom esophageal varices developed, five (four in the timolol group and one in the placebo group) had concomitant gastric varices (three had junctional and two had fundal varices).

† The total number in each group reflects the number of patients who did not reach a primary end point (66 in the timolol group and 63 in the placebo group).

‡ The reasons for transplantation were hepatocellular carcinoma in four patients, decompensated cirrhosis in two patients, and acute-on-chronic liver failure in one patient.

§ In both patients, the reason for transplantation was hepatocellular carcinoma.

¶ Four deaths were related to infection followed by renal or liver dysfunction, one was due to hepatocellular carcinoma, and five were unrelated to liver disease. Ascites, encephalopathy, or both developed in 8 of the 10 patients who died.

‖ Six deaths were related to infection followed by renal dysfunction, three were due to hepatocellular carcinoma, two were due to liver failure, and four were unrelated to liver disease. Ascites, encephalopathy, or both developed in 8 of the 15 patients who died.

** Treatment failure was defined by the occurrence of the primary end point (varices and variceal hemorrhage), transplantation, or death.

RESULTS

A total of 213 patients underwent randomization: 108 were assigned to receive timolol, and 105 to receive placebo (110 at the Barcelona center, 52 at the Connecticut center, 26 at the London center, and 25 at the Boston center). The median time from screening endoscopy to randomization was 29 days (range, 8 to 105). As shown in Table 1, the baseline characteristics were similar in the two groups. There were no significant differences between groups in the proportion of patients with alcohol-induced cirrhosis or in the HVPG. The median Child–Pugh score was 5 (range, 5 to 9; scores can range from 5 to 15, with higher scores indicating more severe liver disease). The median HVPG was 11 mm Hg (range, 6 to 25), with 63 percent of the patients having an HVPG of at least 10 mm Hg. The median duration of follow-up was 54.9 months (range, 0 to 99.4).

The median daily dose of timolol was 10.8 mg

(range, 1.25 to 80.0) in the timolol group, and the median daily dose of the timolol placebo was 12.9 mg (range, 1.25 to 80.0) in the placebo group (according to titration). The dose had to be reduced in 29 patients (26 in the timolol group vs. 3 in the placebo group, P<0.001), and the study medication was withdrawn prematurely in 46 patients (25 in the timolol group vs. 21 in the placebo group, P=0.62).

Adherence to treatment was considered adequate if the pill count showed more than 70 percent adherence; this degree of adherence was achieved in 86 patients in the timolol group (80 percent) and 88 patients in the placebo group (84 percent).

END POINTS

The rates of the primary and secondary end points and treatment failure are shown in Table 2. A total of 84 patients reached the primary end point of varices or variceal bleeding: 42 of 108 patients in the timolol group and 42 of 105 patients in the placebo group (39 percent vs. 40 percent, P=0.89) (Table 2 and Fig. 1). The rates of the primary end point (both overall and for the timolol group) did not differ significantly when patients who had a reduction in the dose or stopped treatment were compared with those who did not have a reduction in the dose or discontinue treatment (data not shown). Hepatocellular carcinoma, which was not considered an end point of the study, occurred in eight patients in the timolol group and six patients in the placebo group.

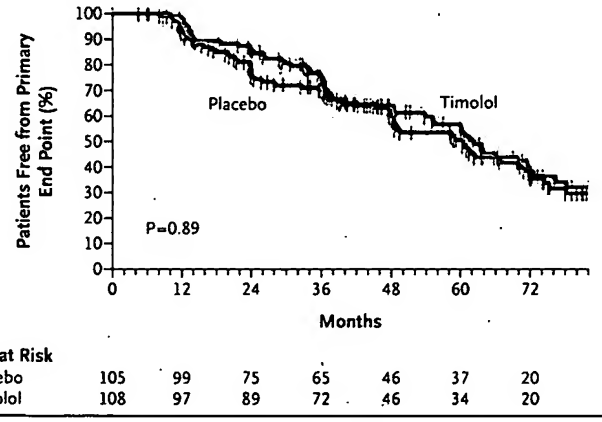

Figure 1. Kaplan–Meier Estimates of the Percentages of Patients Who Did Not Reach the Primary End Point of Varices or Variceal Bleeding.

Cumulative percentages of patients who did not reach the primary end point at 12, 24, 36, and 60 months were 91 percent, 86 percent, 79 percent, and 60 percent, respectively, in the timolol group and 97 percent, 82 percent, 78 percent, and 57 percent, respectively, in the placebo group.

A comparison of the 84 patients who reached the primary end point with the 129 patients who did not reach the primary end point revealed that the following baseline variables differed at a P value of less than 0.1: the Child–Pugh score, the white-cell count, the aspartate aminotransferase level, the alanine aminotransferase level, and the HVPG. On Cox regression analysis, a baseline HVPG of 10 mm Hg or more (P=0.005) and an elevated aspartate aminotransferase level (P=0.007) were independently predictive of reaching the primary end point.

ADVERSE EVENTS

The incidence of moderate or severe adverse events was higher in the timolol group than in the placebo group (48 percent [52 patients] vs. 32 percent [34 patients], P=0.02). Serious adverse events considered probably related to study medication occurred in 20 patients (18 percent) in the timolol group (7 had bradycardia, as defined by a heart rate of less than 50 beats per minute; 5 had severe fatigue; 4 had wheezing or shortness of breath, 2 had syncope; and 1 each had intermittent claudication and impotence) and in 6 patients (6 percent) in the placebo group (1 each had impotence, hypotension, depression, heart failure, nodal rhythm, and bronchospasm) (P=0.006). None of the complications were fatal.

HEMODYNAMICS

Except at baseline, the heart rate was significantly lower in the timolol group than in the placebo group throughout the study (Fig. 2A). The average reduction in the heart rate from baseline was 17 percent in the timolol group. Conversely, the HVPG did not differ significantly between groups during the study (Fig. 2B).

At baseline, an HVPG of 10 mm Hg or more was associated with a significantly higher incidence of the primary end point, as shown in Figure 3A. HVPG measurements were repeated at one year in 154 patients (72 in the timolol group and 82 in the placebo group). As compared with baseline values, the HVPG decreased by a median of 1.45 mm Hg among patients in the timolol group, as compared with a decrease of only 0.5 mm Hg among patients in the placebo group (P=0.07); the decrease in the latter group was due solely to a drop in wedge pressure. Reductions in the HVPG of more than 10 percent (Fig. 3B), more than 15 percent, and more than 20 percent were all associated with a significantly lower incidence of the primary end point. More important, a decrease in the HVPG of more

than 10 percent, more than 15 percent, or more than 20 percent from baseline occurred more frequently in the timolol group (53 percent, 43 percent, and 33 percent, respectively) than in the placebo group (38 percent, 24 percent, and 19 percent, respectively), and these differences were significant (P=0.04, P=0.01, and P=0.04, respectively). Conversely, an increase in the HVPG by more than 10 percent also correlated with an increased likelihood of reaching the end point (Fig. 3C). However, there were no significant differences between groups in the increases in HVPG.

DISCUSSION

In this placebo-controlled study, treatment with a nonselective beta-blocker, timolol, did not prevent gastroesophageal varices in unselected patients with cirrhosis and portal hypertension and was associated with an increased number of adverse effects. A previous French study of the prevention of varices showed that, in patients without varices or with small varices, the development of large varices was more frequent among propranolol-treated patients than among patients who received placebo. Most of the patients had small varices, and significant differences were confined to this subgroup of patients.[8] In contrast, a placebo-controlled study that consisted of patients with small varices found a lower rate of variceal enlargement in patients treated with nadolol.[9]

We used timolol, a potent nonselective beta-blocker,[10] and as shown in patients with essential hypertension,[11] once-daily dosing was sufficient to maintain the reduction in heart rate for at least 24 hours. Moreover, the fact that timolol has a higher affinity for both β_1- and, particularly, β_2-adrenergic receptors than do propranolol and nadolol[10,12] is important, since β_2-adrenergic–receptor blockade is an important target in the reduction of portal pressure.[2] In a study of acute effects, timolol decreased HVPG as effectively as did propranolol or nadolol.[13] However, there are differences in the acute and chronic portal-pressure–reducing effects of beta-blockers,[14,15] which have been ascribed to differences in receptor blockade (β_1-adrenergic receptors are involved in the acute effect and β_2-adrenergic receptors in the chronic effect).[15] Our negative results may have been partially due to the inclusion of patients with an early stage of cirrhosis and thus a milder splanchnic and systemic hyperdynamic circulatory state, a major factor in the maintenance of portal hypertension and the main target of the action of beta-blockers.

The average decrease in heart rate in the timolol group was 17 percent. This reduction is smaller than the range of 20 to 26 percent (median, 24 percent) reported in studies of beta-blockers in the primary prophylaxis of variceal hemorrhage[16-24] and is probably due to the lower baseline heart rate (median, 73 beats per minute) in our study than in primary-prophylaxis studies of patients with varices (median, 80 beats per minute)[9,17-25] or secondary-prophylaxis studies of patients with varices (median, 84 beats per minute).[26-29] However, the absolute heart rate achieved is a better indicator of beta-blockade than the percent reduction in heart rate,[30] and the absolute heart rate in our study during timolol therapy (62 beats per minute) was similar

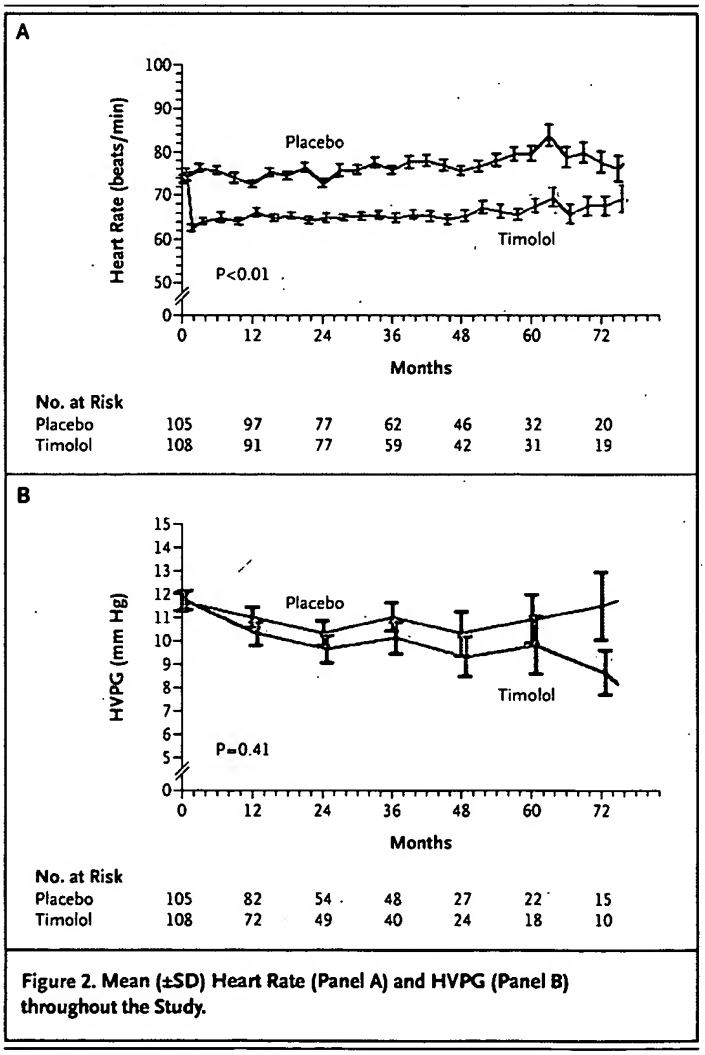

Figure 2. Mean (±SD) Heart Rate (Panel A) and HVPG (Panel B) throughout the Study.

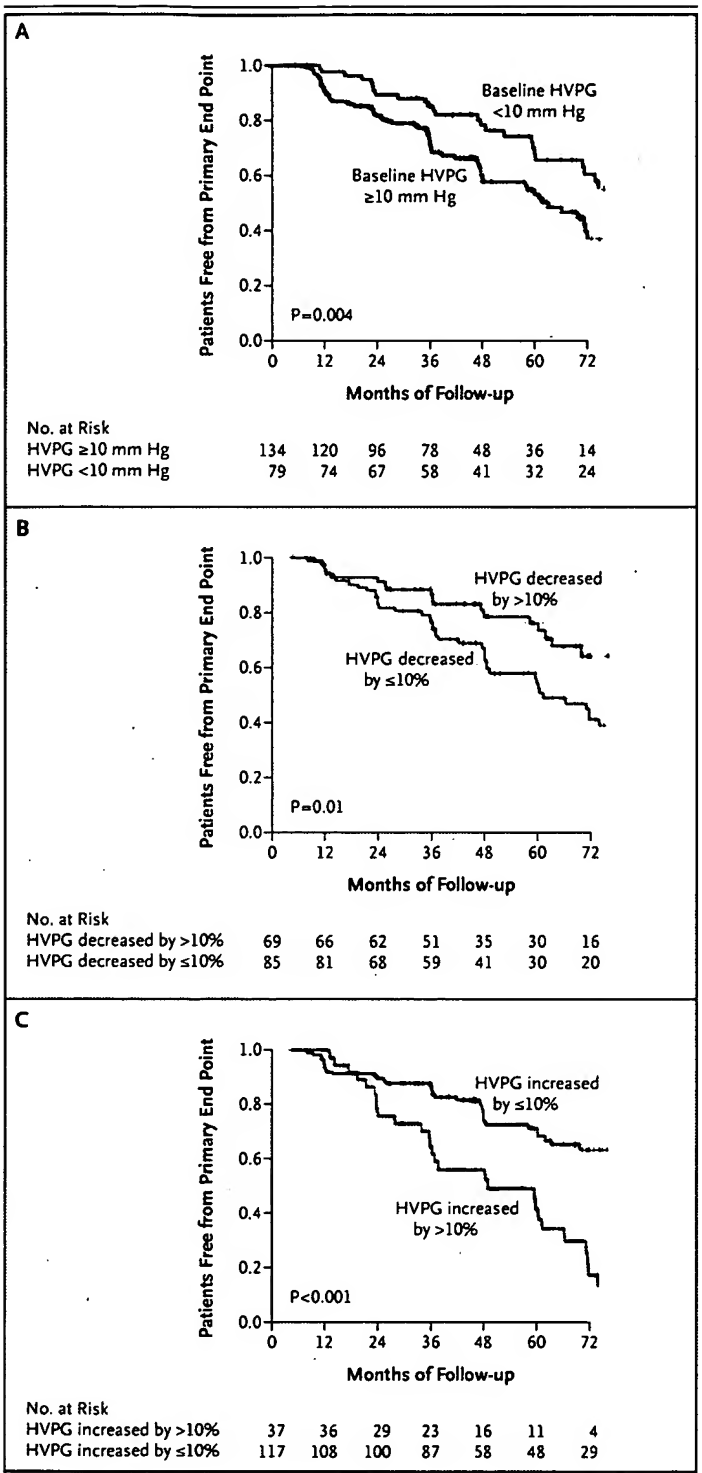

Figure 3. Probability of Remaining Free of the Primary End Point of Varices or Variceal Bleeding, According to the HVPG at Baseline (Panel A) and the Presence or Absence of Either a Decrease in HVPG by More Than 10 Percent (Panel B) or an Increase in HVPG by More Than 10 Percent (Panel C) at One Year.

to the median heart rate of 60 beats per minute among patients receiving beta-blockers in previous studies.[17-24]

The lack of an overall significant change in the HVPG may partly explain our negative results. Although it is possible that positive results could have been obtained if the dose of timolol had been higher, drug intolerance limited our ability to increase the dose further in this group of patients with compensated cirrhosis, most of whom were reluctant to tolerate even minimal side effects.

A major finding of our study was the effect of baseline HVPG on outcomes. The rate of the primary end point was significantly lower among patients with a baseline HVPG of less than 10 mm Hg than among patients with a baseline HVPG of at least 10 mm Hg. This finding supports the definition of clinically significant portal hypertension as an HVPG of at least 10 mm Hg.[31] In fact, the baseline HVPG was the strongest independent predictor of the development of varices.

We confirmed the importance of lowering portal pressure shown in previous studies of patients with more advanced cirrhosis.[29,32-35] We found that reductions in the HVPG of more than 10 percent from baseline were related to a significant decrease in the rate of the primary end point. An important finding was that more patients in the timolol group than in the placebo group had these favorable HVPG responses, indicating that timolol had a beneficial effect, but one that was not sufficient to tip the balance in favor of beta-blockers. Conversely, we also found that increases in portal pressure were associated with the development of varices, although timolol apparently had no ability to prevent this increase in HVPG.

In conclusion, even though the role of nonselective beta-blockers in preventing variceal hemorrhage in patients who already have varices is well established, we found that nonselective beta-adrenergic blockers did not prevent varices in patients with cirrhosis and portal hypertension. The use of beta-blockers cannot be widely recommended in this population because of its association with an increased incidence of serious side effects. However, even in this population of patients with compensated cirrhosis, we have confirmed the predictive value of baseline HVPG levels and of a subsequent reduction in the HVPG by more than 10 percent, the latter of which should be the goal in the pharmacologic prevention of gastroesophageal varices.

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